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AN ECOLOGICAL STUDY OF PARASITES OF SOME NORTH CAROLINA SALAMANDERS

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INTRODUCTION

The relations between salamanders and their parasites and correlations which may exist between habitat and degree of infestation are problems on which little information has been available. The present study is presented as a contribution toward an understanding of these problems. Phylogenetically salamanders are representatives of a group of animals that are transitional between aquatic and terrestial vertebrates. Each family, in fact, may show within itself varying degrees of this transition. Some forms are wholly aquatic, others wholly terrestrial, while others are truly amphibious. Such a condition renders salamanders admirable subjects for study.

In the course of this investigation about a thousand salamanders have been examined to secure data on the parasitic fauna living on or in these hosts. This work has been conducted in North Carolina throughout every month for the period of a year. Under natural conditions there is rarely a single individual salamander which does not harbor at least one or more species of parasites somewhere in its body. The parasites that have been encountered belong to the following groups: Protozoa, Trematoda (flukes), Cestoda (tapeworms), Nematoda (roundworms), Acanthocephala (spiny-headed worms), and Acarina (mites).

Literature

A study of the literature indicates that with the exception of very few writers comparatively little examination of North American salamanders for their parasites has been undertaken. Short papers, describing new species or physiological experiments on particular salamander parasites, form the major part of the work on this subject.

The first record of protozoa inhabiting salamanders was made by Tobey (1906, 1906a) who studied the trypanosomes of the newt, *Triturus v. viridescens*. Intestinal protozoa were recorded by Metcalf (1912), Swezy (1915), Fulton (1923), Tanabe (1926, 1926a), and Woodhead (1928). Nigrelli (1929, 1929a, 1930) found a new sporozoan, *Dactylosoma jahni*, in the erythrocytes of Triturus. Leidy (1851, 1856), Stafford (1900, 1902, 1903, 1905), Chandler (1923), and Holl (1928, 1928b) have made the most important contributions to the study of the trematode parasites of salamanders. Few instances of tapeworm infestation in salamanders have been found. LaRue (1909, 1911a, 1914, 1914a) notes hosts harboring proteocephalids. Thomas (1927), Canavan (1928), and Zeliff (1932) have also reported the occurrence of cestodes in salamanders. Numerous workers have

examined nematodes parasitic in salamanders. Among these writers, the contributions of Holl (1928a), Harwood (1932), Walton (1930, 1930a, 1933, 1935), and Canavan (1931) are the most important. Van Cleave (1915, 1919, 1931) is the only one who has carefully examined the acanthocephalan parasites of salamanders. Howard (1915) reports a mollusc that attacks the gills of Necturus.

The literature on the ecology of salamander parasites and of general surveys of these hosts for their parasites is even more meagre than that on the individual species. Hegner (1921, 1929), Nigrelli (1929b), Holl (1932), Mann (1932), and Pearse (1932) have contributed to this study.

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METHODS

Collection of hosts.—The majority of salamanders collected were obtained by turning over logs and stones and by dipping in ponds and streams with a net. Specimens were placed in small cloth bags with damp moss or leaves. In this condition they could be transported for several days with little mortality. If time did not permit an immediate examination for parasites, the animals were placed in a cold room where the temperature remained at about 4°C. They could be safely kept thus for some time as long as the bags in which they were confined were moist.

Examination of hosts.—An animal was placed under a compound binocular dissecting microscope and examined for external parasites. Brain and spinal cord were then pithed. A slit through the heart was made to obtain blood for fresh slides and smears. It was found that this was the only way enough blood could be obtained for satisfactory results. Fresh blood was placed on a slide with physiological saline and studied for at least ten

minutes. The body cavity was opened by a slit lateral to the mid-ventral line and examined. Diplostomula in the body cavity were more easily and thoroughly removed by shaking the opened animal in a beaker of normal saline. After such treatment, further examination under the binocular microscope was made. All organs were observed *in situ* and then removed to a glass plate. Rectal contents were placed on a slide with saline for study of live protozoa. By adding a drop of Lugol's iodine to the slide the flagella of protozoa were easily observed. The various organs were dissected under the microscope and any parasites found were placed in Syracuse watch glasses in normal saline. The brain and various muscles were teased apart and studied.

Killing and Preserving.—Blood, containing protozoa in the red cells, was smeared on clean slides and allowed to dry. Intestinal protozoa were killed by placing them on slides smeared with albumin and then covered with Schaudinn's fixative. After a few minutes these were transferred to 50 per cent alcohol. Worms were thoroughly washed and freed of mucus before killing. Nematodes were fixed in a straightened condition by dropping them into hot 70 per cent alcohol. They were then preserved in alcohol plus a few drops of glycerine. It was found best to kill Capillaria in cold Bouin's fluid as heat causes this delicate form to shrivel. The majority of trematodes were killed by placing them on a slide under a cover-glass and drawing Conant's fixative underneath by means of filter-paper. This method flattens the worms uniformly, without undue pressure. Cestodes were killed in the same way, or stretched across a glass plate and brushed with a camel's-hair brush dipped in Conant's fixative. The worms were left in the fixative for one-half to four hours. They were then washed with several changes of 50 per cent alcohol and transferred to 70 per cent alcohol for preservation. Acanthocephala must be placed in water until they expand and extrude their probosces, and then killed by dropping into hot 70 per cent alcohol. Acarina were also fixed by dropping them into hot 70 per cent alcohol.

Staining and Mounting.—Blood protozoa were stained in Wright's blood stain, washed and dried. No cover-glass was needed. Intestinal protozoa were stained with iron-haematoxylin and mounted in damar. Most of the helminth material was stained and mounted *in toto*, since usually the general anatomy could be discerned from such mounts. In nearly all cases entire collections were mounted, instead of a mere sampling from each vial, to study specific variations. Nematodes were mounted temporarily in glycerine. The heads of some were cut off and mounted in glycerine-jelly to present an *en face* view. Trematodes were stained in alum-cochineal, cestodes and acanthocephalans in Ehrlich's haematoxylin. All were mounted in damar. Mites were cleared in turpinol and mounted unstained in damar.

SUMMARY OF HOSTS EXAMINED

The following list presents a summary of the number of each species of salamanders examined:

Salamander	*No. examined*
1. *Ambystoma maculatum* (Shaw)	17
2. *Ambystoma opacum* (Gravenhorst)	137
3. *Cryptobranchus alleganiensis* (Daudin)	1
4. *Desmognathus fuscus fuscus* (Raf.)	219
5. *Desmognathus ochrophaeus carolinensis* Dunn	13
6. *Desmognathus ochrophaeus ochrophaeus* (Cope)	3
7. *Desmognathus phoca* (Matthes)	16
8. *Desmognathus quadramaculatus* (Holbrook)	46
9. *Eurycea bislineata cirrigera* (Green)	1
10. *Eurycea bislineata wilderae* Dunn	11
11. *Eurycea gutto-lineata* (Holbrook)	20
12. *Gyrinophilus porphyriticus danielsi* (Blatchley)	1
13. *Plethodon cinereus* (Green)	74
14. *Plethodon glutinosus* (Green)	119
15. *Plethodon metcalfi* Brimley	18
16. *Plethodon yonahlossee* Dunn	3
17. *Pseudotriton montanus montanus* (Baird)	3
18. *Pseudotriton ruber ruber* (Sonnini)	12
19. *Triturus viridescens viridescens* (Raf.)	296
Total Number of Examinations	1,010

The lack of balance between the numbers of different species examined is chiefly a reflexion of their relative abundance. The newt *(Triturus v. viridescens)* is probably the most abundant salamander in the habitats studied and in consequence more have been examined than any other species. The dusky salamander *(Desmognathus f. fuscus)* is evidently extremely abundant in certain localities and a large number of individuals were collected. The marbled salamander *(Ambystoma opacum)* and the slimy salamander *(Plethodon glutinosus)* are about equally abundant in their respective habitats. On the other hand the number of examinations for some species was regrettably low. Practically all of the common salamanders in North Carolina have been covered in this study.

ECOLOGY OF HOSTS EXAMINED

LOCALITIES AND HABITS

North Carolina is one of the most varied states east of the Rockies as far as its ecological features are concerned (Metcalf and Wells, 1926). The elevation ranges from sea level to areas above 6,000 feet. The state may be divided into three natural physiographic regions: the Coastal Plain, the Piedmont Plateau, and the Blue Ridge Mountains.

The Coastal Plain consists of sand dunes, bay lands, cypress swamps, savannas, and pine woods. Attempts made to collect salamanders from this region were unsuccessful. *Amphiuma means* Garden, *Siren lacertina* Linnaeus, and *Necturus m. maculosus* (Raf.) are known to occur there. Only two specimens of *Plethodon glutinosus* (Green) and one of *Triturus v. viridescens* (Raf.) were found.

A majority of the salamanders used in this study were collected in Durham and Orange counties in the lower Piedmont area. This region is characterized by pine woods and valleys covered with deciduous forests in which the dominant trees are beech, maple, tulip, hickory, and oak. Several type habitats were selected and may be designated as follows: Settling Pond, or Nancy Rhodes Pond, and vicinity; Duke Forest; New Hope Creek; Buchanan's Pond; and the artificial ponds at Lakeview (Moore County).

The Settling Pond is about four hundred yards from the Eno River. It is about a half a mile long and fifty yards wide, varying in depth from two to ten feet. It is over forty years old and is constantly being filled with sediment. Emergent vegetation is abundant around the sides. The banks are covered with a mixed deciduous and coniferous forest of yellow pine, maple, and oak. The source of water is from two brooks which empty into the pond at the south end. The overflow takes place at a dam at the opposite end. A distinct thermal stratification occurs in summer that is probably absent in winter. The brooks are characterized by exposed rocks, muddy bottoms, pools, and currents. A thick layer of dead leaves and twigs covers the bottom most of the time. Rocks and logs offer good protection for amphibian fauna. Little emergent vegetation is present, but the banks are overgrown with brush. The width is about four feet where the brooks empty into the pond and dwindles down to a foot or so near the source. The source of water is from springs and drainage from surrounding hills. Both pond and brooks are abundantly supplied with aquatic invertebrate life consisting chiefly of insects, crayfishes, ostracods, snails, earthworms, amphipods, and spiders. Species of salamanders caught there and along the banks are *Desmognathus f. fuscus* (Raf.), *Eurycea gutto-lineata* (Holbrook), *Pseudotriton m. montanus* (Baird), *P. r. ruber* (Sonnini), *Ambystoma maculatum* (Shaw), *Plethodon glutinosus* (Green), and *Triturus v. viridescens* (Raf.).

The Duke Forest is characterized by low hills with growths of pine, maple, oak, hickory, elm, and beech, with a thick carpet of leaf litter. Fallen logs, stones, vegetation, and numerous springs offer refuge for salamanders. Insects, snails, centipedes, and earthworms are abundant. Salamanders collected here are *Plethodon glutinosus* (Green), *P. cinereus* (Green), *Ambystoma maculatum* (Shaw), and *Eurycea bislineata cirrigera* (Green).

That part of the New Hope Creek from which salamanders were collected is the low ground area along the Chapel Hill Road (North Carolina

Highway No. 54) near Durham, North Carolina. Large rotting logs lie thickly scattered over the river bottom among dense vegetation. The river winds in and out, leaving many ox-bow and cut-off ponds that become practically dry in July and August. Crayfishes, snails, ostracods, insects, earthworms, and centipedes afford excellent sustenance for salamanders. *Ambystoma opacum* (Gravenhorst) was collected here throughout the year. and occasionally, *Plethodon glutinosus* (Green), *Desmognathus f. fuscus* (Raf.), and *Eurycea b. cirrigera* (Green).

Buchanan's Pond is located just off the Piny Mountain Road in Orange County. It is very shallow, filled with dense shrubbery and trees. The muddy bottom is covered with dead leaves and algae. The pond covers about half an acre and is fed by springs. Crayfishes, insects, and small clams furnish food for large numbers of amphibians present. *Triturus v. viridescens* (Raf.) was found here in many developmental stages.

About one mile south of Vass, Moore County, North Carolina, many small temporary ponds are found on either side of the highway (Lakeview). These are filled with water most of the time to a depth of about four feet, but become practically dry during July and August. A dense mass of algae, moss, dead leaves, and pine needles covers a muddy bottom. The banks are lined with shrubs, pines and oaks. *Triturus v. viridescens* (Raf.) was found here throughout the year.

A large number of salamanders were taken from the Pisgah National Forest in the Blue Ridge Mountains. This forest consists of 90,000 acres of the upper watershed of the French Broad River, in Transylvania, Buncombe, and Henderson counties (Metcalf and Wells, 1926). It is mostly deciduous interspersed with coniferous forest. Lake Powhatan and neighboring streams, about eight miles north of Asheville, harbor many species of salamanders. Numbers of species were also collected from Grandfather Mountain, Linville, and vicinity. This area is around 5,500 feet above sea-level. Forage animals, serving as food for salamanders, are snails, insects, crayfishes, earthworms, and centipedes. *Desmognathus f. fuscus* (Raf.), *D. o. ochrophaeus* (Cope), *D. o. carolinensis* Dunn, *D. phoca* (Matthes), *D. quadramaculatus* (Holbrook), *Eurycea bislineata wilderae* Dunn, *E. guttolineata* (Holbrook), *Gyrinophilus porphyriticus danielsi* (Blatchley), *Plethodon cinereus* (Green), *P. glutinosus* (Green), *P. metcalfi* Brimley, *P. yonahlossee* Dunn, and *Triturus v. viridescens* (Raf.) were collected from this area.

Nineteen species of hosts were examined in this study. It has been found convenient to assign these to four broad groups: those that are found primarily in water, *aquatic;* those that are usually aquatic but do migrate to land, terrestro-*aquatic;* those that usually live on land but do migrate to water, *terrestro*-aquatic; and those that rarely visit water, *terrestrial.* In the

following notes on the habits of each species, the range has been taken from Stejneger and Barbour's (1933) check list.

Cryptobranchus alleganiensis (Daudin): *Aquatic.*

Range: Western New York, Ohio River and tributaries, and southward to Georgia and Louisiana.

Only one specimen of this species was examined. It had been in the laboratory for some time which may account for its lack of parasites and stomach contents. Cryptobranchus is apparently restricted to rivers and streams in mountainous districts. Little is known of its breeding habits. It feeds on worms, fishes, and crayfishes.

Triturus viridescens viridescens (Raf.): *Aquatic.*

Range: Eastern North America, Hudson Bay to Texas, west to Illinois, Michigan, Missouri, Oklahoma, and southeastern Kansas.

This salamander is the most aquatic of those studied by the writer, excepting Cryptobranchus. Specimens were always collected by dipping them out of the water. In the various localities where *T. v. viridescens* was collected, usually individuals were found near the edge of a stream or pond, never in very deep water. The eggs of this species are laid attached to leaves in the bottom of ponds during spring. The metamorphosed young pass their life on land as a red "eft" in some localities. They remain in this stage for about a year and then return to water as adults. Much doubt is thrown on the necessity of passing through the eft stage. Numerous specimens in this stage have been found in Connecticut and Massachusetts, while only two have been collected in North Carolina by the writer. The chief foods of this newt are aquatic insects, earthworms, snails, crayfishes, ostracods, and organic débris.

Desmognathus fuscus fuscus (Raf.): Terrestro-*aquatic.*

Range: St. John's River, New Brunswick to northwestern Florida, Mississippi, and Illinois, Manitoba?

Desmognathus f. fuscus is terrestrial to the extent that it often lives in and upon moist earth where it mates and lays its eggs, and where the first few days of larval life are spent (Wilder, 1913). The larvae reach shallow water, remain there for a year, and then take up a semi-terrestrial life. The eggs are laid from the first of June to the last of August, and hatch about six weeks after being deposited. Transformation takes place in May or June. In North Carolina this salamander was found in and along streams, under logs, stones, dead leaves and other débris. It is swift, burrows extensively, and may often be found with its head protruding from beneath protective objects. Food consists chiefly of aquatic and terrestrial insects, insect larvae, earthworms, centipedes, crayfishes, snails, spiders, ostracods, and organic débris.

Desmognathus phoca (Matthes): Terrestro-*aquatic*.

Range: Appalachian Mountains from Pennsylvania southward to Toccoa, Georgia, and Newport, Kentucky.

This species is essentially aquatic. Individuals were caught in deep pools, along banks, and under overlying rocks. Some were seen crawling over the bottom. Eggs were found in the latter part of August (Dunn 1926) attached to the edge of a stone in swift-running water. *D. phoca* very closely resembles *D. quadramaculatus*, both in appearance and breeding habits. Stomach contents consisted of earthworms, crayfishes, aquatic insects, ostracods, and snails.

Desmognathus quadramaculatus (Holbrook): Terrestro-*aquatic*.

Range: Virginia to Georgia, in the mountains; 2,000-6,000 feet.

D. quadramaculatus is much the most aquatic species of this genus in North Carolina. Individuals have been found swimming strongly against a current, or across pools, rarely on land. Dunn (1926), however, has seen them running along branches overhanging a stream. The eggs are laid near banks under stones, away from swift currents. Food, as observed in stomach contents, consists of insects, earthworms, tadpoles, ostracods, snails, amphipods, and organic débris.

Eurycea bislineata wilderae Dunn: Terrestro-*aquatic*.

Range: The Southern Blue Ridge region from White Top Mountain, Virginia, south through North Carolina, South Carolina, and Tennessee, to Rabun and Gilmer Counties, Georgia.

The habitat coincides with that of *Desmognathus f. fuscus*. Dunn (1926) says that eggs were found hatching in the middle of July. They were attached to the underside of a stone in a brook. Stomach contents consisted of insects, ostracods, and mud.

Gyrinophilus porphyriticus danielsi (Blatchley): Terrestro-*aquatic*.

Range: Southern section of the Blue Ridge Mountains in western North Carolina and eastern Tennessee. Perhaps South Carolina and Georgia.

Only a single specimen of this species was captured, and this in the same haunts as *Desmognathus f. fuscus*, *D. phoca*, etc., in the mountains. Dunn (1926) collected larvae in rocky streams where they were hiding under submerged objects. The breeding habits apparently agree closely with those of *D. f. fuscus* and Eurycea.

Pseudotriton montanus montanus (Baird): Terrestro-*aquatic*.

Range: New York, Pennsylvania, southern Ohio to western Georgia, Kentucky, and Tennessee.

Specimens were caught in the same habitat with *Desmognathus f. fuscus*, under logs in and near streams. Two were captured under an old tin can in a swampy meadow. None was found far from water. Food consists of crayfishes, snails, earthworms, ostracods, and organic débris.

Pseudotriton ruber ruber (Sonnini): Terrestro-*aquatic*.

Range: From Albany County, New York, to northern Florida, westward to Ohio, Kentucky, Tennessee, Alabama, and northern Mississippi.

Some of these salamanders were found in a spring, the rest in situations with *Desmognathus f. fuscus*. Larvae of several different sizes were collected. This indicates, as Dunn (1926) suggests, that this salamander takes at least two years in the larval stage. It resembles closely *D. f. fuscus* and *P. m. montanus* in its breeding activities and general habitat. Stomach contents consisted of snails, young Desmognathus, crayfishes, and ostracods.

Desmognathus ochrophaeus carolinensis Dunn: *Terrestro*-aquatic.

Range: Beverly, West Virginia, to Gwinnett County, Georgia, in the mountains.

D. o. carolinensis, in its habits, is quite like that of the Plethodons and seems as terrestrial as they. Some specimens, however, were taken in and near a small stream. The majority of them were found under rocks in damp places. Eggs have been found in July (Dunn, 1926) in rotten logs on the banks of a stream. Food consists of insects, snails, centipedes, and earthworms.

Desmognathus ochrophaeus ochrophaeus (Cope): *Terrestro*-aquatic.

Range: Clinton County, New York, to Garrett County, Maryland, west to Columbus, Ohio, in the Allegheny Plateau and the Appalachian Valley.

This species is very closely related to *D. o. carolinensis*, both in breeding habits and general habitat. It was found under logs, stones, and other objects, in the mountains in or near streams. Stomach contents showed a diet of earthworms, insects, crayfishes, snails, and organic debris.

Eurycea bislineata cirrigera (Green): *Terrestro*-aquatic.

Range: North Carolina south to northwestern Florida, west through Alabama and southern Louisiana; western Tennessee.

This salamander was found in the same habitat in which *Desmognathus f. fuscus* was collected, under logs and rocks in and near a stream. Brimley (1896) says that it breeds from December to March. Larvae first appear in May. Food was found to consist of insects, snails, and algae.

Eurycea gutto-lineata (Holbrook): *Terrestro*-aquatic.

Range: Fairfax County, Virginia, to Liberty County, Georgia, west to Alabama, Mississippi, and Louisiana; western Tennessee. Region includes southern Coastal Plain and Piedmont, southern Blue Ridge, and extreme southern part of the Appalachian Valley.

Individuals of this species were obtained by overturning rocks above a stream. None was caught in water, though frequently seen there. Brimley (1896) found specimens containing eggs in November. Insect remains, earthworms, snails, and mud were found in stomachs.

Ambystoma maculatum (Shaw): *Terrestro*-aquatic.

Range: Nova Scotia west to Wisconsin, southward to Georgia (possibly Florida) and Texas.

Ambystoma maculatum was collected from areas closely adjoining swampy regions, under logs. Often it was found under the same log with *Plethodon*

glutinosus. It breeds in spring after the first warm rain following a thaw. Eggs are laid in water; larvae hatch there and remain for about five months. Larvae were caught under dead leaves at the bottom of streams. Food consists of earthworms, centipedes, insects and crayfishes.

Ambystoma opacum (Gravenhorst): *Terrestro*-aquatic.

Range: From Massachusetts to Georgia, west to Louisiana and Texas, Mississippi basin, north to Arkansas, Missouri, Indiana, and Illinois.

This salamander is terrestrial to the extent that it lives on land except for a short period of its larval life. The larvae are aquatic during winter and emerge in spring. Adults and immature individuals were found at all seasons under rotting logs, usually near ponds. Occasionally, some were found under bark of stumps, or even in tunnels made by the wood-boring beetle, *Passalus cornutus* Fabricius. At no time were adults observed in water. The chief foods of adults are ants and beetles. Other items in their diet were insects, millipedes, centipedes, earthworms, snails, crayfishes, and organic debris. Contents of larval stomachs revealed a high percentage of crustacean food, chiefly ostracods.

Plethodon cinereus (Green): *Terrestrial.*

Range: Cape Breton Island west to Fort William, Ontario, south to Dallas, Georgia; Missouri, and Arkansas.

Both in the mountains and in the Durham area, this salamander was collected high above streams in oak-hickory forests. It was usually found under bark lying on the ground, often in groups of two or three. Dunn (1926) notes that eggs are laid in grape-like clusters, supported by a pedicle and attached to the under sides of logs or stones, usually beneath the surface of the ground. Food consists of snails, insects, earthworms, and centipedes.

Plethodon glutinosus (Green): *Terrestrial.*

Range: Southern New York to Wisconsin, south to northern Florida, the Gulf States to Texas, and Missouri.

Plethodon glutinosus was always found in habitats away from water, usually under fallen logs. Occasionally, some were caught under pine, but the majority were taken from logs of deciduous trees. This salamander agrees closely with *P. cinereus* in its habits. Noble (1931) states that eggs are laid deep under ground in walls of caves, old mouse burrows, etc. The chief article of diet is beetles. Other foods are insects, spiders, earthworms, snails, and millipedes.

Plethodon metcalfi Brimley: *Terrestrial.*

Range: Southwestern Virginia south through western North Carolina, northern Georgia and northeastern Alabama. It apparently occupies all the ranges of the Southern Blue Ridge except the Great Smokies and the Nantahalas.

This salamander was taken in the mountains in the same habitat with *P. cinereus.* It occurs under fallen bark and logs, and even between the bark and wood of large fallen limbs some distance from the ground. Stomach contents showed insect remains, snails, isopods, and organic debris.

Plethodon yonahlossee Dunn: *Terrestrial.*

Range: Wooded areas in the mountains near Linville, North Carolina, and in southwestern Virginia. Dr. I. E. Gray and the writer collected this salamander in the Unaka National Forest, northeastern Tennessee.

The habitat resembles closely that of *P. glutinosus*. *P. yonahlossee* is quite rare, having been reported only a few times, primarily near the type locality (Dunn, 1926). Dunn says that it is probably the most primitive of the whole genus Plethodon. Centipedes, ants, beetles, and organic débris were found in stomachs.

PARASITES OF HOSTS EXAMINED

The parasites found in the hosts studied are presented in the following list. The name of the parasite, percentage of infestation, average number of parasites per host (except for protozoa), and the region of infestation are given. At the time this study was made, the writer considered the trematode species *Manodistomum occultum* Stafford, 1905, *Plagitura parva* Stunkard, 1933, and *Plagitura salamandra* Holl, 1928 synonymous. Subsequent study has shown that these may be valid species (see Appendix 1). Consequently, percentage of infestation, etc., for these parasites are listed under the heading Plagitura sp. Tables 5-11 give the seasonal distribution of the parasites.

Ambystoma maculatum (Shaw)

Protozoa

Cytamoeba bacterifera Labbe, 1894; 35.2; erythrocytes.
Euglenamorpha hegneri Wenrich, 1923; 5.8; rectum.
Hexamitus intestinalis (Dujardin, 1841); 29.4; rectum.
Prowazekella longifilis Alexieff, 1912; 47.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 58.8; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 88.2; 18.1; intestine.
Diplostomulum ambystomae Rankin and Hughes, 1937; 11.7; 16.2; body cavity.

Nematoda

Spirurid cysts; 52.9; 15.4; mesenteries, intestinal wall.

Acarina

Hannemania dunni Sambon, 1928; 41.1; 7.1; skin.

Ambystoma opacum (Gravenhorst): Larvae

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 31.2; blood.
Eutrichomastix batrachorum Dobell, 1909; 6.2; rectum.
Hexamitus intestinalis (Dujardin, 1841); 37.5; rectum.

Prowazekella longifilis Alexieff, 1912; 37.5; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 37.5; rectum.

Trematoda

Diplostomulum ambystomae Rankin and Hughes, 1937; 18.7; 19.7; body cavity.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 31.2; 0.62; intestine.

Ambystoma opacum (Gravenhorst): Adults

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 9.09; blood.
Cytamoeba bacterifera Labbe, 1894; 0.09; erythrocytes.
Eimeria ranarum (Labbe); 5.7; intestine.
Eutrichomastix batrachorum Dobell, 1909; 10.7; rectum.
Haptophrya michiganensis Woodhead, 1928; 6.6; intestine and rectum.
Hexamastix batrachorum Alexieff, 1912; 1.6; rectum.
Hexamitus intestinalis (Dujardin, 1841); 6.6; rectum.
Prowazekella longifilis Alexieff, 1912; 34.7; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 65.2; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 82.6; 17.3; intestine.
Diplostomulum ambystomae Rankin and Hughes, 1937; 69.4; 148.7; body cavity.
Gorgoderina bilobata Rankin, 1937; 16.5; 0.71; bladder.
Megalodiscus temperatus (Stafford, 1905) Harwood, 1932; 0.8; 0.008; rectum.
Plagitura sp.; 0.8; 0.22; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 57.0; 6.81; intestinal mucosa.
Cosmocercoides dukae (Holl, 1928) Wilkie, 1930; 28.9; 1.2; rectum.
Filaria sp.; 0.8; 0.008; eye.
Spirurid cysts; 6.6; 0.38; stomach wall.

Acarina

Hannemania dunni Sambon, 1928; 6.6; 1.0; skin.

Cryptobranchus alleganiensis (Daudin)

Protozoa

Prowazekella longifilis Alexieff, 1912; 100.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 100.0; rectum.

Desmognathus fuscus fuscus (Raf.): Larvae; Durham

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 13.6; blood.
Eutrichomastix batrachorum Dobell, 1909; 9.09; rectum.
Hexamitus batrachorum Swezy, 1915; 18.1; rectum.
Hexamitus intestinalis (Dujardin, 1841); 9.09; rectum.
Prowazekella longifilis Alexeiff, 1912; 63.6; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 45.4; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 4.9; 0.09; intestine.

Cestoda

Proteocephalid cysts; 31.8; 1.9; intestinal wall.

Nematoda

Physaloptera sp.; 4.9; 0.18; stomach.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 4.9; 0.09; intestine.

Desmognathus fuscus fuscus (Raf.): Adults; Durham

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 21.0; blood.
Cytamoeba bacterifera Labbe, 1894; 28.3; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 33.1; rectum.
Hexamastix batrachorum Alexieff, 1912; 7.8; rectum.
Hexamitus batrachorum Swezy, 1915; 17.4; rectum.
Hexamitus intestinalis (Dujardin, 1841); 19.2; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 20.5; rectum.
Prowazekella longifilis Alexieff, 1912; 62.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 69.7; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 22.2; 0.98; intestine.
Gorgoderina bilobata Rankin, 1937; 2.4; 0.05; bladder.
Megalodiscus intermedius (Hunter, 1930) Harwood, 1932; 0.6; 0.06; rectum.
Megalodiscus temperatus (Stafford, 1905) Harwood, 1932; 0.6; 0.1; rectum.
Metacercariae; 41.5; 6.8; encysted under tongue, muscles, etc.
Phyllodistomum solidum Rankin, 1937; 5.4; 0.07; bladder.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1937; 13.2; 0.6; intestine.

Crepidobothrium plerocercoids; 3.01; 0.12; intestine.
Proteocephalid cysts; 5.4; 0.18; mesenteries, intestinal wall.

Nematoda

Cosmocercoides dukae (Holl, 1928) Wilkie, 1930; 6.0; 0.1; rectum.
Physaloptera sp.; 2.4; 0.07; stomach.
Spirurid cysts; 13.8; 0.5; mesenteries and intestinal wall.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 5.4; 0.07; intestine.
Cysts; 3.01; 0.04; body cavity.

Desmognathus fuscus fuscus (Raf.): Mountains

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 45.4; blood.
Cytamoeba bacterifera Labbe, 1894; 30.3; erythrocytes.
Eutrichomasitx batrachorum Dobell, 1909; 30.3; rectum.
Haptophrya michiganensis Woodhead, 1928; 12.1; intestine.
Hexamastix batrachorum Alexieff, 1912; 12.1; rectum.
Hexamitus batrachorum Swezy, 1915; 42.4; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 21.2; rectum.
Prowazekella longifilis Alexieff, 1912; 66.6; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 33.3; 1.12; intestine.
Diplostomulum desmognathi Rankin, 1937; 24.2; 4.3; body cavity.
Gorgoderina bilobata Rankin, 1937; 9.0; 0.12; bladder.
Metacercariae; 24.2; 5.9; intestinal wall, muscles.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 15.1; 0.6; intestine.
Crepidobothrium plerocercoids; 6.0; 0.24; intestine.
Proteocephalid cysts; 30.3; 2.33; intestinal wall.

Nematoda

Capillaria inequalis Walton, 1935; 3.03; 0.09; intestinal mucosa.
Omeia papillocauda Rankin, 1937; 3.03; 0.09; rectum.
Oxyuris magnavulvaris Rankin, 1937; 48.4; 0.9; rectum and intestine.
Spirurid cysts; 1.7; 0.1; mesentery.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 3.03; 0.09; intestine.
Cysts; 3.03; 0.06; body cavity.

Desmognathus ochrophaeus carolinensis Dunn

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 7.6; blood.
Eutrichomastix batrachorum Dobell, 1909; 7.6; rectum.
Hexamastix batrachorum Alexieff, 1912; 23.08; rectum.
Hexamitus intestinalis (Dujardin, 1841); 7.6; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 7.6; intestine.
Prowazekella longifilis Alexieff, 1912; 30.7; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 38.4; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 7.6; 0.07; intestine.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 15.3; 0.15; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 7.6; 0.07; intestinal mucosa.
Oxyuris magnavulvaris Rankin, 1937; 23.08; 0.3; rectum.

Desmognathus ochrophaeus ochrophaeus (Cope)

Protozoa

Prowazekella longifilis Alexieff, 1912; 33.3; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Nematoda

Capillaria inequalis Walton, 1935; 7.6; 0.07; intestinal mucosa.

Desmognathus phoca (Matthes)

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 37.5; blood.
Cytamoeba bacterifera Labbe, 1894; 50.0; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 6.3; rectum.
Haptophrya michiganensis Woodhead, 1928; 6.3; intestine.
Hexamastix batrachorum Alexieff, 1912; 25.0; rectum.
Hexamitus batrachorum Swezy, 1915; 31.2; rectum.
Hexamitus inetstinalis (Dujardin, 1841); 6.3; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 31.2; rectum.
Prowazekella longifilis Alexieff, 1912; 50.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 62.5; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 18.7; 0.37; intestine.
Diplostomulum desmognathi Rankin, 1937; 31.2; 12.7; body cavity.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 6.2; 0.06; intestine.
Crepidobothrium plerocercoids; 6.2; 0.06; intestine.

Nematoda

Omeia papillocauda Rankin, 1937; 6.2; 0.06; rectum.
Oxyuris magnavulvaris Rankin, 1937; 25.0; 0.62; rectum.
Spirurid cysts; 6.2; 0.25; intestinal wall.

Desmognathus quadramaculatus (Holbrook)

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 6.5; blood.
Cytamoeba bacterifera Labbe, 1894; 34.7; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 36.9; rectum.
Hexamastix batrachorum Alexieff, 1912; 21.7; rectum.
Hexamitus batrachorum Swezy, 1915; 32.6; rectum.
Hexamitus intestinalis (Dujardin, 1841); 10.8; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 17.3; rectum.
Prowazekella longifilis Alexieff, 1912; 65.2; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 34.7; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 8.6; 0.52; intestine.
Diplostomulum desmognathi Rankin, 1937; 8.6; 2.04; body cavity.
Metacercariae; 10.8; 4.8; muscles, mesenteries.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 17.3; 0.91; intestine.
Crepidobothrium plerocercoids; 17.3; 0.41; intestine.
Proteocephalid cysts; 6.5; 0.13; mesenteries.

Nematoda

Capillaria inequalis Walton, 1935; 2.1; 0.06; intestinal mucosa.
Omeia papillocauda Rankin, 1937; 13.04; 0.3; rectum and intestine.
Oxyuris magnavulvaris Rankin, 1937; 15.2; 0.32; rectum.
Spirurid cysts; 6.5; 0.5; intestinal wall.

Acanthocephala

Cysts; 0.02; 0.06; body cavity.

Eurycea bislineata cirrigera (Green)

Protozoa

Prowazekella longifilis Alexieff, 1912; 100.0; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 100.0; 5.0; intestine.

Cestoda

Proteocephalid cysts; 100.0; 10; intestinal wall.

Eurycea bislineata wilderae Dunn

Protozoa

Cytamoeba bacterifera Labbe, 1894; 9.09; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 9.09; rectum.
Haptophrya michiganensis Woodhead, 1928; 9.09; intestine.
Hexamastix batrachorum Alexieff, 1912; 9.09; rectum.
Hexamitus batrachorum Swezi, 1915; 18.1; rectum.
Prowazekella longifilis Alexieff, 1912; 18.1; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 18.1; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 18.1; 1; intestine.

Cestoda

Proteocephalid cysts; 36.3; 1; mesentery.

Nematoda

Oxyuris magnavulvaris Rankin, 1937; 27.2; 0.63; rectum and intestine.

Eurycea gutto-lineata (Holbrook): Durham

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 16.6; blood.
Cytamoeba bacterifera Labbe, 1894; 16.6; erythrocytes.
Prowazekella longifilis Alexieff, 1912; 33.3; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 100.0; rectum.

Trematoda

Gorgoderina tenua Rankin, 1937; 33.3; 0.5; bladder.
Plagitura sp.; 33.3; 0.33; intestine.

Cestoda

Proteocephalid cysts; 50.0; 1.0; intestinal wall.

Acarina

Hannemania dunni Sambon, 1928; 16.6; 1.0; skin.

Eurycea gutto-lineata (Holbrook): Mountains

Protozoa

Eutrichomastix batrachorum Dobell, 1909; 35.7; rectum.
Haptophrya michiganensis Woodhead, 1928; 21.4; intestine.
Hexamastix batrachorum Alexieff, 1912; 42.8; rectum.
Hexamitus batrachorum Swezy, 1915; 14.2; rectum.
Prowazekella longifilis Alexieff, 1912; 35.7; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 7.1; 0.14; intestine.

Nematoda

Oxyuris magnavulvaris Rankin, 1937; 64.2; 2.5; rectum and intestine.
Spirurid cysts; 7.1; 1.07; mesenteries and stomach wall.

Gyrinophilus porphyriticus danielsi (Blatchley)

Protozoa

Hexamastix batrachorum Alexieff, 1912; 100.0; rectum.
Prowazekella longifilis Alexieff, 1912; 100.0; rectum.

Nematoda

Omeia papillocauda Rankin, 1937; 100.0; 2; intestine.

Plethodon cinereus (Green): Durham

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 11.5; blood.
Cytamoeba bacterifera Labbe, 1894; 7.6; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 3.4; rectum.
Prowazekella longifilis Alexieff, 1912; 23.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 96.1; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 3.4; 0.03; intestine.

Nematoda

Cosmocercoides dukae (Holl, 1928) Wilkie, 1930; 3.4; 0.19; rectum.

Plethodon cinereus (Green): Mountains

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 31.2; blood.
Cytamoeba bacterifera Labbe, 1894; 8.3; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 31.2; rectum.
Hexamastix batrachorum Alexieff, 1912; 12.5; rectum.

Hexamitus batrachorum Swezy, 1915; 22.9; rectum.
Hexamitus intestinalis (Dujardin, 1841); 4.1; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 22.9; rectum.
Prowazekella longifilis Alexieff, 1912; 60.4; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 62.5; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 47.9; 2.14; intestine.

Cestoda

Crepidobothrium plerocercoids; 8.3; 0.31; intestine.

Nematoda

Oxyuris magnavulvaris Rankin, 1937; 2.08; 0.02; rectum.

Plethodon glutinosus (Green): Durham

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 6.8; blood.
Cytamoeba bacterifera Labbe, 1894; 2.2; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 25.0; rectum.
Haptophrya gigantea Maupas, 1879; 3.4; intestine.
Haptophrya michiganensis Woodhead, 1928; 11.3; intestine.
Hexamastix batrachorum Alexieff, 1912; 4.5; intestine.
Hexamitus batrachorum Swezy, 1915; 30.6; rectum.
Hexamitus intestinalis (Dujardin, 1841); 18.1; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 6.8; rectum.
Prowazekella longifilis Alexieff, 1912; 71.5; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 42.0; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 63.6; 5.3; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 17.0; 0.48; intestinal mucosa.
Cosmocercoides dukae (Holl, 1928) Wilkie, 1930; 29.5; 1.01; rectum.
Spirurid cysts; 12.6; 2.4; stomach wall.

Acarina

Hannemania dunni Sambon, 1928; 20.4; 3.2; skin.

Plethodon glutinosus (Green): Mountains

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 3.4; blood.
Cytamoeba bacterifera Labbe, 1894; 6.9; erythrocytes.

Eutrichomastix batrachorum Dobell, 1909; 24.1; rectum.
Haptophrya michiganensis Woodhead, 1928; 6.9; intestine.
Hexamastix batrachorum Alexieff, 1912; 31.0; rectum.
Hexamitus batrachorum Swezy, 1915; 3.4; rectum.
Hexamitus intestinalis (Dujardin, 1841); 3.4; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 24.1; rectum.
Prowazekella longifiilis Alexieff, 1912; 72.4; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 41.3; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 55.1; 9.8; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 3.4; 0.03; intestinal mucosa.
Oswaldocruzia pipiens Walton, 1929; 3.4; 0.03; intestine.
Oxyuris magnavulvaris Rankin, 1937; 3.4; 0.06; rectum.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 3.4; 0.06; intestine.

Plethodon glutinosus (Green): Coastal Plain

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 100.0; blood.
Prowazekella longifilis Alexieff, 1912; 50.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 100.0; intestine.

Trematoda

Brachycoelium hospitale Stafford, 1900; 100.0; 1.0; intestine.

Plethodon metcalfi Brimley

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 5.5; blood.
Eutrichomastix batrachorum Dobell, 1909; 22.2; rectum.
Hexamastix batrachorum Alexieff, 1912; 11.1; rectum.
Hexamitus intestinalis (Dujardin 1841); 5.5; rectum.
Karotomorpha swezi (Grassi, 1926) Trav's, 1934; 22.2; rectum.
Prowazekella longifilis Alexieff, 1912; 55.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 50.0; 2.38; intestine.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 27.2; 0.27; intestine.
Crepidobothrium plerocercoids; 11.1; 0.11; intestine.

Plethodon yonahlossee Dunn

Protozoa

Cytamoeba bacterifera Labbe, 1894; 66.6; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 66.6; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 66.6; rectum.
Prowazekella longifilis Alexieff, 1912; 66.6. rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 33.3; 0.66; intestine.

Nematoda

Oxyuris magnavulvaris Rankin, 1937; 33.3; 0.33; rectum.

Pseudotriton montanus montanus (Baird)

Protozoa

Haptophrya michiganensis Woodhead, 1928; 33.3; intestine.
Prowazekella longifilis Alexieff, 1912; 100.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 100.0; rectum.

Trematoda

Allocreadium pseudotritoni Rankin, 1937; 66.6; 1.0; intestine.
Gorgoderina bilobata Rankin, 1937; 33.3; 1.0; bladder.

Nematoda

Physaloptera sp.; 33.3; 1.0; stomach.

Acarina

Hannemania dunni, Sambon, 1928; 33.3; 14.8; skin.

Pseudotriton ruber ruber (Sonnini): Larvae

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 44.4; blood.
Cytamoeba bacterifera Labbe, 1894; 11.1; erythrocytes.
Eutrichomastix batrachorum Dobell, 1909; 22.2; rectum.
Hexamastix batrachorum Alexieff, 1912; 22.2; rectum.
Hexamitus intestinalis (Dujardin, 1841); 22.2; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 11.1.; rectum.

Prowazekella longifilis Alexieff, 1912; 77.7; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Trematoda

Allocreadium pseudotritoni Rankin, 1937; 55.5; 1.66; intestine.
Metacercariae; 11.1; 0.22; intestinal wall.

Cestoda

Proteocephalid cysts; 11.1; 0.11; intestinal wall.

Pseudotriton ruber ruber (Sonnini): Adults

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 33.3; blood.
Cytamoeba bacterifera Labbe, 1894; 100.0; erythrocytes.
Prowazekella longifilis Alexieff, 1912; 100.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 33.3; rectum.

Trematoda

Allocreadium pseudotritoni Rankin, 1937; 33.3; 0.33; intestine.
Brachycoelium hospitale Stafford, 1900; 33.3; 6.0; intestine.
Gorgoderina bilobata Rankin, 1937; 33.3; 1.0; bladder.

Cestoda

Crepidobothrium cryptobranchi (LaRue, 1914) Meggett, 1927; 33.3; 0.33; intestine.

Triturus viridescens viridescens (Raf.): Coastal Plain

Protozoa

Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 100.0; rectum.

Triturus viridescens viridescens (Raf.): Duke Forest; eft stage

Trematoda

Brachycoelium hospitale Stafford, 1900; 100.0; 1.0; intestine.

Triturus viridescens viridescens (Raf.): Buchanan's Pond; larvae

Protozoa

Hexamastix batrachorum Alexieff, 1912; 4.0; rectum.
Hexamitus intestinalis (Dujardin, 1841); 8.0; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 4.0; rectum.

Trematoda

Metacercariae; 56.0; 35.6; intestinal wall.
Plagitura sp.; 88.0; 7.1; intestine.

Nematoda

Camallanus sp.; 4.0; 0.04; intestine.

Triturus viridescens viridescens (Raf.): Buchanan's Pond; adults

Protozoa

Eutrichomastix batrachorum Dobell, 1909; 77.2; rectum.
Hexamastix batrachorum Alexieff, 1912; 22.7; rectum and intestine.
Hexamitus batrachorum Swezy, 1915; 20.4; rectum.
Hexamitus intestinalis (Dujardin, 1841); 18.1; rectum.
Prowazekella longifilis Alexieff, 1912; 81.8; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 40.9; rectum.

Trematoda

Megalodiscus temperatus (Stafford, 1905) Harwood, 1932; 47.7; 2.4; rectum.
Plagitura sp.; 68.1; 7.02; intestine.

Nematoda

Camallanus sp.; 2.2; 0.02; intestine.
Capillaria inequalis Walton, 1935; 86.3; 4.9; intestinal mucosa.
Cosmocercoides dukae (Holl, 1928) Wilkie, 1930; 15.9; 0.31; rectum.
Spirurid cysts; 59.0; 12.5; stomach wall.

Triturus viridescens viridescens (Raf.): Settling Pond.

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 59.2; blood.
Cytamoeba bacterifera Labbe, 1894; 11.1; erythrocytes.
Hexamitus batrachorum Swezy, 1915; 11.1; rectum.
Prowazekella longifilis Alexieff, 1912; 88.8; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 44.4; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 11.1; 0.48; intestine.
Gorgoderina bilobata Rankin, 1937; 7.3; 0.07; bladder.
Megalodiscus temperatus (Stafford, 1905) Harwood, 1932; 25.9; 2.25; rectum.
Metacercariae; 14.8; 1.5; intestinal wall.
Plagitura sp.; 40.7; 2.7; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 70.3; 3.9; intestinal mucosa.
Spirurid cysts; 29.6; 3.2; stomach wall.

Acanthocephala

Acanthocephalus acutulus Van Cleave, 1931; 3.7; 0.11; intestine.

Triturus viridescens viridescens (Raf.): Lakeview

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 27.4; blood.
Cytamoeba bacterifera Labbe, 1894; 11.5; erythrocytes.
Entamoeba ranarum Grassi, 1879; 2.6; rectum.
Eutrichomastix batrachorum Dobell, 1909; 25.6; rectum.
Hexamastix batrachorum Alexieff, 1912; 5.3; rectum.
Hexamitus batrachorum Swezy, 1915; 23.8; rectum.
Hexamitus intestinalis (Dujardin, 1841); 3.4; rectum.
Karotomorpha swezi (Grassi, 1926) Travis, 1934; 2.6; rectum.
Myxobolus conspicuus Kudo, 1919; 2.6; muscles at base of head.
Prowazekella longifilis Alexieff, 1912; 54.7; rectum.
Tritrichomonas augusta (Alexieff, 1911) Kofoid, 1920; 70.8; rectum.

Trematoda

Brachycoelium hospitale Stafford, 1900; 19.4; 1.3; intestine.

Cestoda

Proteocephalid cysts; 0.8; 0.008; intestinal wall.

Nematoda

Capillaria inequalis Walton, 1935; 36.2; 0.99; intestinal mucosa.
Spirurid cysts; 66.6; 6.2; intestinal wall.

Triturus viridescens viridescens (Raf.): Lake Powhatan; eft stage

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 100.0; blood.

Trematoda

Brachycoelium hospitale Stafford, 1900; 100.0; 11; intestine.

Nematoda

Oxyuris magnavulvaris Rankin, 1937; 100.0; 3; rectum.

Triturus viridescens viridescens (Raf.): Lake Powhatan; adults

Protozoa

Cryptobia borreli (Laveran and Mesnil, 1901); 11.9; blood.
Cytamoeba bacterifera Labbe, 1894; 14.2; erythrocytes.
Entamoeba ranarum Grassi, 1879; 1.1; rectum.
Euglenamorpha hegneri Wenrich, 1923; 1.1; rectum.
Eutrichomastix batrachorum Dobell, 1909; 25.2; rectum.
Hexamastix batrachorum Alexieff, 1912; 8.3; rectum and intestine.

Hexamitus batrachorum Swezy, 1915; 30.9; rectum.
Hexamitus intestinalis (Dujardin, 1841); 4.6; rectum.
Nyctotherus cordiformis (Ehrenberg, 1838); 2.3; rectum.
Prowazekella longifilis Alexieff, 1912; 67.8; rectum.

TABLE 1. Percentage of Infestation According to Habitat. Protozoa. Hosts Range from Aquatic to Terrestrial in Habitat Preference.

Salamander Hosts	Cryptobia	Cytamoeba	Haptophrya michiganensis	Haptophrya gigantea	Nyctotherus	Euglenamorpha	Eutrichomastix	Hexamastix	Hexamitus batrachorum	Hexamitus intestinalis	Karotomorpha	Prowazekella	Tritrichomonas	Entamoeba	Eimeria	Myxobolus
Triturus larvae, Buchanan's	—	—	—	—	—	—	—	4.0	—	8.0	—	—	4.0	—	—	—
Triturus adults, Buchanan's	—	—	—	—	—	—	77.2	22.7	20.4	18.1	—	81.8	40.9	—	—	—
Triturus adults, Durham	59.2	11.1	—	—	—	—	—	—	11.1	—	—	88.8	44.4	—	—	—
Triturus adults, Lakeview	27.4	11.5	—	—	—	—	25.6	5.3	23.8	3.5	2.6	54.7	70.8	2.6	—	2.6
Triturus adults, Mountains	11.9	14.2	—	—	2.3	1.1	25.2	8.3	30.9	4.6	—	67.8	—	1.1	—	—
Desmognathus f. fuscus, larvae, Durham	13.6	—	—	—	—	—	9.09	—	18.1	9.09	—	63.6	45.4	—	—	—
Desmognathus f. fuscus, adults, Durham	21.0	28.3	—	—	—	—	33.1	7.8	17.4	19.2	20.5	62.0	69.8	—	—	—
Desmognathus f. fuscus, adults, Mountains	45.4	30.3	12.1	—	—	—	30.3	12.1	42.4	—	21.2	66.6	33.3	—	—	—
D. phoca	37.5	50.0	6.3	—	—	—	6.3	25.0	31.2	6.3	31.2	50.0	62.5	—	—	—
D. quadramaculatus	6.5	34.7	—	—	—	—	36.9	21.7	32.6	10.8	17.3	65.2	34.7	—	—	—
Pseudotriton r. ruber, larvae	44.4	11.1	—	—	—	—	22.2	22.2	—	22.2	11.1	77.7	33.3	—	—	—
Eurycea gutto-lineata, Durham	16.6	16.6	—	—	—	—	—	—	—	—	—	33.3	100.0	—	—	—
Eurycea gutto-lineata, Mountains	—	—	21.4	—	—	—	35.7	42.8	14.2	—	—	—	35.7	—	—	—
Ambystoma maculatum	—	35.2	—	—	—	5.8	—	—	—	29.4	—	47.0	58.8	—	—	—
A. opacum, larvae	31.2	—	—	—	—	—	6.2	—	—	37.5	—	37.5	37.5	—	—	—
A. opacum, adults	9.09	9.09	6.6	—	—	—	10.7	1.6	—	6.6	—	34.7	65.2	—	5.7	—
Plethodon cinereus, Durham	11.5	7.6	—	—	—	—	3.4	—	—	—	—	23.0	96.1	—	—	—
Plethodon cinereus, Mountains	31.2	8.3	—	—	—	—	31.2	12.5	22.9	4.1	22.9	60.4	62.5	—	—	—
P. glutinosus, Durham	6.8	2.2	11.3	3.4	—	—	25.0	4.5	30.6	18.1	6.8	71.5	42.0	—	—	—
P. glutinosus, Mountains	3.4	6.9	6.9	—	—	—	24.1	31.0	3.4	3.4	24.1	72.4	41.3	—	—	—
P. metcalfi	5.5	—	—	—	—	—	22.2	11.1	—	5.5	22.2	55.0	33.3	—	—	—

Trematoda

Brachycoelium hospitale Stafford, 1900; 25.0; 1.2; intestine.
Plagitura sp.; 59.5; 3.8; intestine.

Nematoda

Capillaria inequalis Walton, 1935; 4.7; 0.04; intestinal mucosa.
Oxyuris magnavulvaris Rankin, 1937; 8.2; 0.17; rectum.
Spirurid cysts; 20.2; 0.52; stomach and intestinal wall.

ECOLOGY OF PARASITES FOUND

PARASITISM AND HABITAT

The species of salamanders examined in this study, as already mentioned, can be arranged in a series that indicates various degrees of preference for aquatic, terrestro-aquatic, and terrestrial habitats. The Plethodons are the only members of this series that have attained a more or less complete freedom from water. The remaining hosts studied require that some part of their life cycles be spent in water.

PROTOZOA

All species of hosts examined were infested to some extent with protozoan parasites. Blood protozoa (Table 1) are not limited to any particular habitat. Triturus from Buchanan's Pond, however, is free from this infestation. This probably may be correlated with the absence of leeches, the intermediate hosts. The fact that terrestrial salamanders harbor blood protozoa indicates that they must occasionally enter the aquatic habitat or that they have retained a larval parasitism. *Eurycea gutto-lineata* in the mountains, collected in the main stream below an infested area is also free from blood protozoa. A sandy and rocky shore and bottom afford little protection for leeches. This condition plus a lower temperature than in the infested area and a certain degree of immunity may account for non-infestation.

Salamanders from Settling Pond (Nancy Rhodes) show a steady decrease from aquatic to terrestrial habitat in infestation with Cryptobia (Fig. 1). Hosts from the mountains, on the other hand, are most heavily infested in the terrestro-aquatic types of environment. Infestation in both mountains and lowland is lowest in terrestrial hosts. Aquatic larvae of *Ambystoma opacum* have a higher percentage of infestation with Cryptobia (31.2) than adults (9.09). On the other hand, larvae of *Desmognathus f. fuscus,* though also aquatic, have a lower percentage of infestation (13.6) than adults (21.0). Size may be an important factor as well as habitat preference of adults. Larvae of *A. opacum* are from three to five times as large as those of *D. f. fuscus.* The larger larvae may be more open to attack by leeches and so become infested, whereas the smaller, more obscure larval hosts may escape. Salamanders from the Durham region, in general, are more heavily infested than those from the mountains.

The highest percentage of infestation with Cytamoeba, both in the mountains and Durham, occurs in terrestro-*aquatic* salamanders (Fig. 1), the lowest in terrestrial. Infestation in the mountains tends to exceed slightly that at Durham. A high percentage of infestation with Cryptobia is usually accompanied by a low one with Cytamoeba, and *vice versa.* The presence of one seems to limit the abundance of the other. When Cytamoeba is the

prevailing parasite the percentage of infestation is never as high as when Cryptobia occupies this position. The large infestations with Cryptobia would indicate that this parasite is in some way dominant to Cytamoeba.

In the mountains, most species of hosts that are terrestro-*aquatic* are as a rule, more heavily infested with both Cryptobia and Cytamoeba than are those that are strictly aquatic or terrestrial. At Durham, aquatic hosts are more heavily infested (Fig. 2).

Parasitism with intestinal ciliates tends to be more or less closely correlated with a terrestrial habitat. *Haptophrya michiganensis* is observed in both mountain and Durham regions. No infestation occurs in aquatic salamanders.

Intestinal flagellates are found commonly in all species of hosts (Table 1). Two flagellates, Prowazekella and Tritrichomonas, are more abundantly represented than others. An interesting habitat correlation has been observed with respect to flagellates from Durham salamanders. Hosts are found in Settling Pond (Triturus), in the neighboring or tributary brook

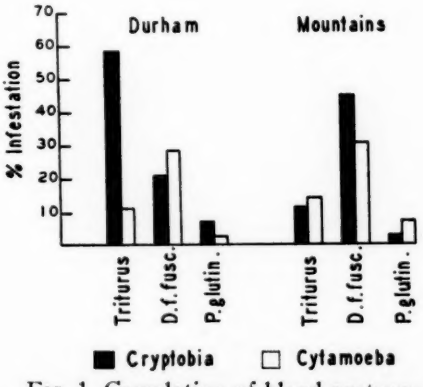

FIG. 1. Correlation of blood protozoa with habitat types, both at Durham and in the mountains, North Carolina.

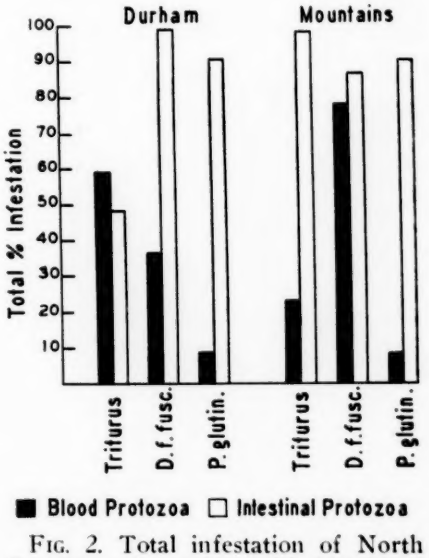

FIG. 2. Total infestation of North Carolina salamanders with blood and intestinal protozoa with respect to type and range of habitat.

(Desmognathus f. fuscus), and in the nearby forest, *(Plethodon glutinosus)*. *D. f. fuscus* is infested with a flagellate fauna that may be arranged in a series of descending order, from high infestation with Tritrichomonas to low with Hexamastix (Fig. 3). Only three of the protozoa occur in the aquatic, where as all are found in the terrestrial host. Except for two cases *(Prowazekella* and *Hexamitus batrachorum)* terrestro-aquatic hosts are the most heavily parasitized. Tritrichomonas and Prowazekella appear to be universally common to various habitats. A succession of flagellate species in

the different habitats beginning with pond, to brook, and to land appears to be present. This correlation does not hold in the mountains (Fig. 4).

The distribution of *Hexamitus batrachorum* at Durham represents a series (Fig. 3) that ranges from terrestrial to aquatic hosts in prevalence. In the mountains this parasite is most abundant in terrestro-*aquatic*, least in terrestrial hosts (Fig. 4). The data also indicate that Eutrichomastix, Hexamastix, and Karotomorpha are associated with land habitats and that brook salamanders become infested with these protozoans during land migrations.

A study of Triturus from several ponds indicates that Buchanan's Pond has a higher percentage of infestation with Eutrichomastix and Hexamastix than do other ponds. Triturus from Lakeview is most heavily infested with different flagellate species, while Settling Pond hosts are least infested. Tritrichomonas is absent from mountain newts, but infests Lakeview hosts most heavily. Karotomorpha is found only at Lakeview.

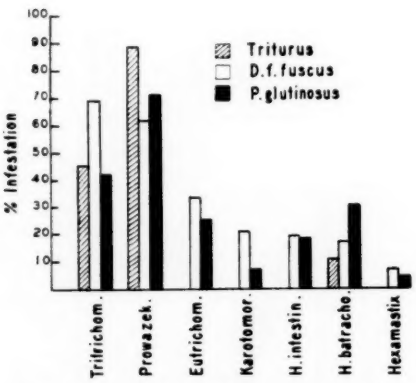

FIG. 3. Distribution of intestinal flagellates in salamanders of representative habitats at Durham, N. C.

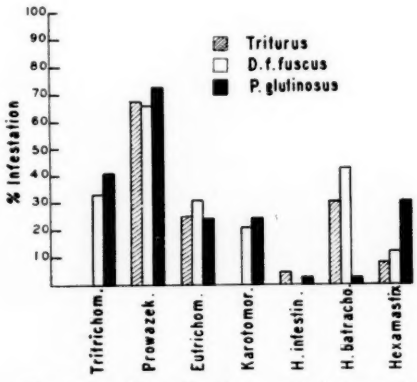

FIG. 4. Distribution of intestinal flagellates in salamanders of representative habitats in the mountains of North Carolina.

Often differences in parasitism are due to host-specificity and conclusions derived from difference of parasites in various habitats on such data might not be justified. This study, however, indicates that all protozoa are not specific for particular hosts, thus substantiating the present conclusions.

A study of total percentage of infestation with blood and intestinal protozoa indicates a few definite correlations (Table 2). Blood protozoa are represented usually by a high percentage in aquatic hosts and low in terrestrial. Salamanders at Settling Pond show this series, but in the mountains they do not. Terrestro-aquatic hosts exhibit much fluctuation with respect to such parasitism, some are highly infested *(Desmognathus f. fuscus)*, others not *(Eurycea b. wilderae)*. Though a host is aquatic, it does not necessarily follow that it shall be heavily parasitized, for terrestro-aquatic salamanders in the mountains have more individuals infested than do aquatic.

Infestation with blood protozoa in Triturus assumes the following series: Settling Pond, Lakeview, Mountains, Buchanan's Pond. Settling Pond is permanent and fed by brooks. The temporary pools at Lakeview dry up in summer. Lake Powhatan, in the mountains, is often drained and is much younger than Settling Pond. Buchanan's Pond is fed by springs and is relatively temporary, depending on the season. The older the habitat, the more likely is higher percentage of infestation. Opportunity for extraneous intermediate hosts, *i. e.*, entrance from brooks, is also probably an important factor.

TABLE 2. Total Percentage of Infestation. Hosts Arranged from Aquatic to Terrestrial in Habitat Preference.

Salamander Hosts	Blood Protozoa	Intestinal Protozoa	Adult Trematoda	Encysted metacercariae	Intestinal flukes	Bladder flukes	Cestoda	Cestode cysts	Nematoda	Nematode cysts
Triturus, larvae, Buchanan's	—	8.0	92.0	56.0	92.0	—	—	—	4.0	—
Triturus, adults, Buchanan's	—	100.0	86.3	—	86.3	—	—	—	88.6	59.0
Triturus, adults, Durham	59.2	48.1	59.2	—	55.5	7.4	—	—	70.3	29.6
Triturus, adults, Lakeveiw	37.8	97.2	16.3	—	16.3	—	—	0.8	42.3	66.6
Triturus, adults, Mountains	23.7	98.5	74.1	—	74.1	—	—	—	17.8	20.2
Desmognathus f. fuscus, larvae, Durham	13.6	77.2	4.5	—	—	—	—	31.8	4.5	—
D. f. fuscus, adults, Durham	36.3	99.3	27.3	43.1	20.5	8.2	19.8	5.4	9.5	13.8
D. f. fuscus, adults, Mountains	78.6	87.5	23.2	24.2	19.8	7.1	21.4	30.3	42.9	1.7
D. phoca	43.7	93.7	18.7	—	18.7	—	12.5	—	31.2	6.2
D. quadramaculatus	34.7	97.8	8.7	10.8	8.7	—	34.7	6.5	26.0	6.5
Eurycea b. wilderae	9.09	54.5	18.1	—	18.1	—	—	36.3	36.3	—
Pseudotriton r. ruber, larvae	55.5	77.7	55.5	11.1	55.5	—	—	11.1	—	—
Eurycea gutto-lineata, Mountains	—	78.5	7.1	—	7.1	—	—	—	64.2	7.1
Ambystoma maculatum	35.2	76.4	88.2	—	88.2	—	—	—	—	52.9
A. opacum, larvae	31.2	37.5	—	—	—	—	—	—	—	—
A. opacum, adults	13.4	80.6	86.5	—	84.0	16.7	—	—	65.5	6.6
Plethodon cinereus, Durham	15.3	96.1	3.4	—	3.4	—	—	—	3.4	—
Plethodon cinereus, Mountains	36.9	91.3	54.7	—	54.7	—	8.6	—	2.1	—
P. glutinosus, Durham	8.6	91.3	60.8	—	60.8	—	—	—	39.1	12.6
P. glutinosus, Mountains	6.8	89.6	62.0	—	62.0	—	—	—	10.3	—
P. metcalfi	5.5	77.7	50.0	—	50.0	—	27.7	—	—	—

Brook and land, rather than aquatic salamanders in Settling Pond have a high degree of parasitism with intestinal protozoa (Fig. 2). This condition may be correlated with a greater variety of parasite species inhabiting terrestrial rather than aquatic hosts. Mountain salamanders exhibit a high degree of infestation with intestinal protozoa (Fig. 2). The percentage of infested newts there is strikingly above that of those in Settling Pond.

TABLE 3. Percentage of Infestation According to Habitat. Trematoda and Cestoda. Upper Figure = % Infestation; Lower = Average Number of Parasites Per Host. Hosts Range from Aquatic to Terrestrial in Habitat Preference.

Salamander Hosts	Allocreadium	Brachycoelium	Diplostomulum ambystomae	Diplostomulum desmognathi	Gorgoderina bilobata	Gorgoderina tenua	Plagitura	Megalodiscus intermedius	Megalodiscus temperatus	Metecercariae	Phyllodistomum	Crepidobothrium cryptobranchi	Crepidobothrium plerocercoids	Proteocephalid cysts
Triturus, larvae Buchanan's	—	—	—	—	—	—	88.0 7.1	—	—	56.0 3.56	—	—	—	—
Triturus, adults, Buchanan's	—	—	—	—	—	—	52.0 2.3	—	31.8 2.5	—	—	—	—	—
Triturus, adults, Durham	—	11.1 0.5	—	—	7.3 0.07	—	40.7 2.7	—	25.9 2.3	14.8 1.5	—	—	—	—
Triturus, adults, Lakeview	—	19.4 1.3	—	—	—	—	—	—	—	—	—	—	—	0.8 0.008
Triturus, adults, Mountains	—	25.0 1.2	—	—	—	—	59.5 3.8	—	—	—	—	—	—	—
Desmognathus f. fuscus, larvae, Durham	—	4.9 0.2	—	—	—	—	—	—	—	—	—	—	—	31.8 1.9
D. f. fuscus, adults, Durham	—	22.2 0.98	—	—	2.4 0.05	—	—	0.6 0.06	0.6 0.1	41.5 6.8	5.4 0.07	13.2 0.6	3.01 0.12	5.4 0.18
D. f. fuscus, adults, Mountains	—	33.3 1.12	—	24.2 4.3	9.0 0.12	—	—	—	—	24.2 5.9	—	15.1 0.6	6.0 0.24	30.3 2.38
D. phoca	—	18.7 0.37	—	31.2 12.7	—	—	—	—	—	—	—	6.2 0.06	6.2 0.06	—
D. quadramaculatus	—	8.6 0.52	—	8.6 2.04	—	—	—	—	—	10.8 4.8	—	17.3 0.91	17.3 0.41	6.5 0.13
Eurycea b. wilderae	—	18.1 1.0	—	—	—	—	—	—	—	—	—	—	—	36.3 1.0
Pseudotriton r. ruber, larvae	55.5 1.66	—	—	—	—	—	—	—	—	11.1 0.22	—	—	—	11.1 0.11
Eurycea gutto-lineata, Durham	—	—	—	—	—	33.3 0.5	33.3 0.33	—	—	—	—	—	—	50.0 1.0
Eurycea gutto-lineata, Mountains	—	7.1 0.14	—	—	—	—	—	—	—	—	—	—	—	—
Ambystoma maculatum	—	88.2 18.1	11.7 16.2	—	—	—	—	—	—	—	—	—	—	—
A. opacum, larvae	—	—	18.7 19.7	—	—	—	—	—	—	—	—	—	—	—
A. opacum, adults	—	82.6 7.3	69.4 148.7	—	16.5 0.72	—	0.8 0.22	—	0.8 0.008	—	—	—	—	—
Plethodon cinereus, Durham	—	3.4 0.03	—	—	—	—	—	—	—	—	—	—	—	—
Plethodon cinereus, Mountains	—	47.9 2.14	—	—	—	—	—	—	—	—	—	—	8.3 0.31	—
P. glutinosus, Durham	—	63.6 5.3	—	—	—	—	—	—	—	—	—	—	—	—
P. glutinosus, Mountains	—	55.1 9.8	—	—	—	—	—	—	—	—	—	—	—	—
P. metcalfi	—	50.0 2.38	—	—	—	—	—	—	—	—	—	27.2 0.27	11.1 0.11	—

Perhaps Triturus goes through a land stage more regularly in the mountains than at Durham. Should this be so, land protozoa would be found in aquatic stages.

TREMATODA

The trematodes observed in salamanders of the various localities studied exhibit a few significant correlations with environment (Table 3). Salamanders living in a terrestro-*aquatic* habitat are parasitized with more species of trematodes than those living in other types. *Desmognathus f. fuscus* is infested with six species, *Triturus v. viridescens* with five, and

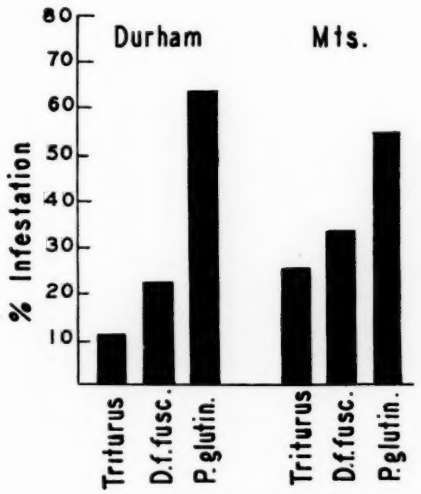

FIG. 5. Correlation of infestation of salamanders with the trematode *Brachycoelium hospitale* with habitat both at Durham and in the mountains, N. C.

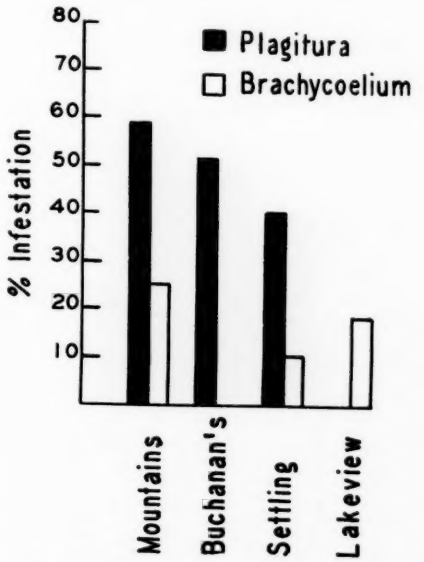

FIG. 6. Comparison of infestation of Triturus from several localities with Plagitura and Brachycoelium. Note apparent correlation of Plagitura with aquatic habitat.

Plethodon glutinosus, P. cinereus and *P. metcalfi* with but one. Salamanders in the lowlands contain a more varied fauna than do those in the mountains. Triturus from Settling Pond harbors five species, whereas this same host from the mountains has but two. *Desmognathus f. fuscus* from Durham is infested with six species, the mountain host with but four. On the other hand, the strictly terrestrial salamanders from both regions harbor but a single species of trematode. The complete absence of other species of flukes from terrestrial hosts is significant. This absence suggests that the assumption of a land habitat with a complete reduction of time spent in water has minimized effectively the chance of infestation with various trematode parasites. Trematodes are probably largely confined to aquatic habitats by their dependence on snails as intermediate hosts.

Brachycoelium hospitale seems to be universally prevalent in all habitats from which salamanders were collected. It occurs in fifteen of the nineteen species of hosts studied. Such a wide-spread distribution is indicative of either a similar distribution of intermediate hosts or of a high degree of adjustment on the part of the parasite to various hosts. The average number of these parasites infesting hosts is greater in *terrestro*-aquatic and terrestrial types than in those from other habitats. The percentage of infestation (Fig. 5) represents a series from low in aquatic to high in terrestrial hosts both at Durham and in the mountains.

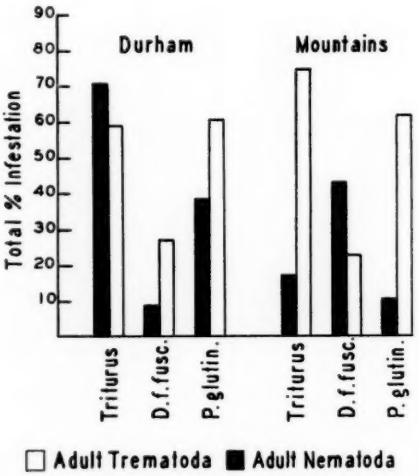

FIG. 7. Comparison of total infestation of salamanders from representative habitats with adult trematodes and nematodes both at Durham and in the mountains, N. C.

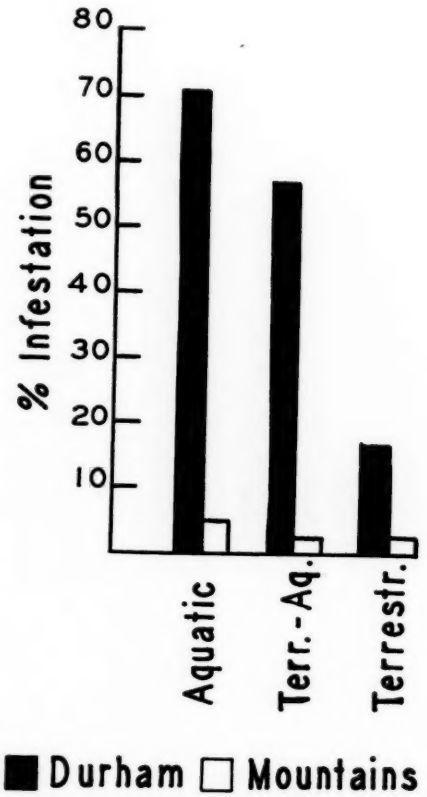

FIG. 8. Distribution of the nematode *Capillaria inequalis*, in representative habitats both at Durham and in the mountains, N. C.

The occurrence of Plagitura sp. is apparently definitely correlated with an aquatic habitat (Fig. 6). Lakeview is the only locality from which Triturus negative for this fluke was taken. The presence of Plagitura in *Eurycea gutto-lineata* and *Ambystoma opacum* may be accidental, the former through its terrestro-aquatic adult habit, the latter through its aquatic larval habit.

Amphistomes are also more or less definitely limited to an aquatic environment. None were found in mountain habitats. The prevalence of en-

cysted larval flukes is significantly correlated with a terrestro-*aquatic* habitat. This correlation is in agreement with the known methods of entrance of cercariae by active penetration. Excysted metacercariae, Diplostomula, are present in the mountains and in the Piedmont area in *terrestro*-aquatic hosts.

Bladder flukes are found primarily in terrestro-*aquatic* salamanders with two exceptions. Triturus from Settling Pond is infested with *Gorgoderina bilobata*, as is adult *Ambystoma opacum*. Phyllodistomum is found only in *Desmognathus f. fuscus*, and *Gorgoderina tenua* only in *Eurycea guttolineata* in Settling Pond. Phyllodistomum and *G. tenua* are apparently limited to the lowland area, while *G. bilobata* occurs also in the mountains.

Total infestation with adult flukes is about equal in the mountains and at Durham. In both areas terrestro-aquatic hosts are less parasitized (Fig. 7), whereas infestation in aquatic and terrestrial hosts is about equal. This condition may be due to the fact that one species of trematodes *(Brachycoelium hospitale)* is predominantly terrestrial, another (Plagitura) aquatic, and that percentage of infestation is transitional in brook hosts.

Limited areas may yield high or low total percentages of infestations with intestinal flukes according to the abundance of particular parasite species. Only 16.3% of newts from Lakeview are infested, as opposed to 86.3% from Buchanan's Pond. The latter is characterized by the prevalence of Plagitura in large numbers and the absence of Brachycoelium. The situation is reversed at Lakeview and the percentage of infestation with Brachycoelium is low.

CESTODA

The most striking correlation observed in relation to cestodes found in this study is the apparent dependence of these helminths on the terrestro-*aquatic* habitat (Table 3). Apparently copepods, the usual intermediate hosts, are prevalent in this type of environment. The fact that a few instances of infestation in terrestrial hosts are encountered indicates that these salamanders occasionally migrate to water, or that insects may serve as intermediate hosts. Adult cestodes and plerocercoids are fairly equally abundant in the mountains and at Durham. The presence of tapeworm cysts is also correlated with the terrestro-*aquatic* habitat. None of the terrestrial hosts is infested, and only 0.8% of the newts from Lakeview.

NEMATODA

Most of the nematode population of salamanders is fairly constant in distribution, as shown in Table 4. It appears that terrestro-aquatic hosts are least parasitized. *Capillaria inequalis* is commonly distributed, occurring most frequently in aquatic salamanders (Fig. 8). It is much more abundant in the Piedmont area, low average numbers per host obtaining in mountain hosts. *Cosmocercoides dukae*, *Omeia papillocauda*, and *Oxyuris magnavulvaris* are apparently definitely limited to particular regions. The first is

TABLE 4. Percentage of Infestation According to Habitat. Nematoda, Acanthocephala, and Acarina. Upper Figure = % Infestation; Lower = Average Number of Parasites Per Host. Hosts Range from Aquatic to Terrestrial in Habitat Preference.

Salamander Hosts	Capillaria	Camallanus	Cosmocercoides	Filaria	Omeia	Oswaldocruzia	Oxyuris	Physaloptera	Spirurid cysts	Acanthocephalus acutulus	Acanthocephalan cysts	Hannemania
Triturus, larvae, Buchanan's	—	4.0 0.04	—	—	—	—	—	—	—	—	—	—
Triturus, adults, Buchanan's	81.5 0.8	2.2 0.02	15.9 0.31	—	—	—	—	—	47.7 8.4	—	—	—
Triturus, adults, Durham	70.3 3.9	—	—	—	—	—	—	—	29.6 3.2	3.7 0.11	—	—
Triturus, adults, Lakeview	36.2 0.99	—	—	—	—	—	—	—	66.6 6.2	—	—	—
Triturus, adults, Mountains	4.7 0.04	—	—	—	—	—	8.2 0.17	—	20.2 0.52	—	—	—
Desmognathus f. fuscus, larvae, Durham	—	—	—	—	—	—	—	4.9 0.18	—	4.9 0.09	—	—
D. f. fuscus, adults, Durham	—	—	6.0 0.1	—	—	—	—	2.4 0.07	13.8 0.5	5.4 0.07	3.01 0.04	—
D. f. fuscus, adults, Mountains	3.03 0.09	—	—	—	3.03 0.09	—	48.4 0.9	—	1.7 0.1	3.03 0.09	3.03 0.06	—
D. phoca	—	—	—	—	6.2 0.06	—	25.0 0.62	—	6.2 0.25	—	—	—
D. quadramaculatus	2.1 0.06	—	—	—	13.0 0.3	—	15.2 0.34	—	6.5 0.5	—	0.02 0.06	—
Eurycea b. wilderae	—	—	—	—	—	—	27.2 0.63	—	—	—	—	—
Pseudotriton r. ruber, larvae	—	—	—	—	—	—	—	—	—	—	—	—
Eurycea gutto-lineata, Durham	—	—	—	—	—	—	—	—	—	—	—	16.6 1.0
Eurycea gutto-lineata, Mountains	—	—	—	—	—	—	64.2 2.5	—	7.1 1.07	—	—	—
Ambystoma maculatum	—	—	—	—	—	—	—	—	52.9 15.4	—	—	41.1 7.1
A. opacum, larvae	—	—	—	—	—	—	—	—	—	31.2 0.62	—	—
A. opacum, adults	57.0 6.81	—	28.9 1.2	0.8 0.008	—	—	—	—	6.6 0.38	—	—	6.6 1.0
Plethodon cinereus, Durham	—	—	3.4 0.19	—	—	—	—	—	—	—	—	—
Plethodon cinereus, Mountains	—	—	—	—	—	—	2.08 0.02	—	—	—	—	—
P. glutinosus, Durham	17.0 0.5	—	29.5 1.0	—	—	—	—	—	12.6 2.4	—	—	20.4 3.2
P. glutinosus, Mountains	3.4 0.03	—	—	—	—	3.4 0.03	3.4 0.06	—	—	3.4 0.06	—	—
P. metcalfi	—	—	—	—	—	—	—	—	—	—	—	—

found only at Durham, while the latter two are found only in the mountains. The occurrence of Cosmocercoides primarily in terrestrial hosts is closely correlated with the life cycle of this parasite. It is oviparous and the eggs must be eaten by the definitive host before development can take place. Omeia and Oxyuris, on the other hand, are viviparous and this fact probably accounts for the more prevalent infestation in terrestro-aquatic hosts. That host-specificity and other biotic factors must be considered is indicated by the number of negative hosts in infested localities.

A study of total infestation with nematodes at Durham (Fig. 7) indicates that aquatic hosts are most heavily infested. This may be correlated with the prevalence of Capillaria in aquatic salamanders. In the mountains terrestro-aquatic hosts have the greatest infestation. Omeia and Oxyuris, specific for the mountains and primarily specific for terrestro-aquatic habitats, probably account for this condition. Aside from this fact, the Durham area is more heavily infested than the mountains.

The distribution of nematode cysts at Durham is correlated with the aquatic habitat. The same obtains for the mountains. Cysts are much more prevalent in Buchanan's Pond and in Lakeview than in other habitats. A limited area may be an important factor in this distribution.

ACANTHOCEPHALA

The prevalence of *Acanthocephalus acutulus* seems significantly linked with an aquatic environment (Table 4). Aquatic larvae of *Ambystoma opacum* are infested, as are several adult species of hosts. The occurrence of *A. acutulus* in a Plethodon is probably accidental. Cysts are restricted to a terrestro-aquatic habitat. A single specimen of *Pomphorhynchus bulbicolli* was found in a newt *(Triturus v. viridescens)* from Leverett, Mass. This parasite has been reported previously only from fish hosts. Its occurrence in a salamander is considered accidental. These parasites are generally associated with a host in all stages of their development, which suggests that infestation is brought about by ingestion of the proper intermediate host. They are equally abundant in mountain and lowland habitats.

ACARINA

Hannemania dunni is apparently limited to lowland hosts from terrestro-aquatic and terrestrial regions (Table 4). No aquatic or mountain salamanders are infested with this larval mite.

SEASONAL VARIATION

Enough salamanders have been examined to give significant results with respect to seasonal periodicity of parasites. Some parasites show marked seasonal variation, others none at all (Tables 5-11). The present study indicates that hosts should be examined over a period of several years before

TABLE 5. Seasonal Infestation of *Desmognathus f. fuscus*, Durham, N. C., November, 1934, to December, 1935. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Number hosts examined	10	10	16	10	11	10	10	9	7	10	9	11	15	9
Ave. length of hosts (m.m.)	111.5	109.3	98.2	110.5	99.2	76.5	115.3	111.4	102.7	108.5	113.2	114.2	104.8	111.2
Total % infest., intestinal Protozoa	100	100	87.5	100	100	100	100	100	100	100	100	100	100	100
Total % infest., adult Metazoa	20.0	20.0	50.0	30.0	27.2	10.0	30.0	66.6	57.1	80.0	77.7	72.7	46.6	77.7
	0.2	0.2	1.5	0.6	1.4	0.1	0.4	3.3	2.0	5.8	4.6	3.4	2.2	2.8
Cryptobia	0	0	81.2	70.0	81.8	0	0	0	0	20.0	11.1	9.09	0	22.2
Cytamoeba	0	80.0	68.7	60.0	63.6	0	0	0	0	30.0	33.3	27.2	6.6	55.5
Eutrichomastix	60.0	20.0	31.2	20.0	63.6	10.0	30.0	55.5	42.8	40.0	33.3	27.2	40.0	55.5
Hexamastix	0	0	0	50.0	0	30.0	10.0	22.2	14.2	0	0	0	0	11.1
Hexamitus batrachorum	0	0	0	0	0	0	0	0	0	60.0	77.7	45.4	60.0	22.2
Hexamitus intestinalis	0	0	0	20.0	9.09	50.0	40.0	22.2	57.1	0	33.3	18.1	46.6	0
Karotomorpha swezi	0	0	0	0	0	40.0	40.0	66.6	42.8	40.0	11.1	45.4	33.3	0
Prowazekella	80.0	40.0	62.5	60.0	90.9	60.0	90.0	66.6	85.7	80.0	55.5	54.5	53.3	100
Tritrichomonas	80.0	100	75.0	80.0	90.9	30.0	70.0	88.8	100	90.0	88.8	63.6	73.3	88.8
Acanthocephalus acutulus	10.0	0	0	0	9.09	0	0	0	0	10.0	0	9.09	13.3	33.3
	0.1	0	0	0	0.18	0	0	0	0	0.1	0	0.18	0.13	0.55
Acanthocephalan cysts	0	0	6.2	30.0	0	0	0	0	0	0	11.1	0	0	0
	0	0	0.18	0.3	0	0	0	0	0	0	0.22	0	0	0
Brachycoelium	0	10.0	18.7	10.0	18.1	0	20.0	55.5	57.1	40.0	11.1	18.1	20.0	22.2
	0	0.2	0.25	0.1	1.27	0	0.3	2.77	2.0	2.2	0.44	0.9	0.26	0.66
Crepidobothrium cryptobranchi	0	0	0	0	0	10.0	0	0	0	50.0	66.6	45.4	20.0	22.2
	0	0	0	0	0	0.1	0	0	0	2.0	3.6	1.7	1.9	0.22
Crepidobothrium plerocercoids	0	0	25.0	10.0	0	0	0	0	0	0	0	0	0	0
	0	0	1.12	0.3	0	0	0	0	0	0	0	0	0	0
Cosmocercoides dukae	10.0	0	0	0	0	0	10.0	22.2	0	20.0	11.1	9.09	0	22.2
	0.1	0	0	0	0	0	0.1	0.55	0	0.4	0.22	0.27	0	0.44
Gorgoderina bilobata	0	10.0	0	0	0	0	0	0	0	0	0	0	0	33.3
	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0.66
Megalodiscus intermedius	0	0	6.2	0	0	0	0	0	0	0	0	0	0	0
	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0
Megalodiscus temperatus	0	0	0	0	0	0	0	0	0	0	0	9.09	0	0
	0	0	0	0	0	0	0	0	0	0	0	0.18	0	0
Metacercariae	10.0	40.0	6.2	0	18.1	60.0	100	88.8	85.7	80.0	66.6	54.5	6.6	44.4
	0.7	2.5	6.0	0	6.87	3.5	9.1	10.1	9.7	9.9	9.7	6.7	0.9	2.1
Phyllodistomum solidum	0	0	0	30.0	0	0	0	0	0	0	22.2	9.09	6.6	22.2
	0	0	0	0.3	0	0	0	0	0	0	0.33	0.18	0.06	0.33
Physaloptera sp.	0	0	0	0	0	0	0	0	0	10.0	0	0	6.6	11.1
	0	0	0	0	0	0	0	0	0	0.2	0	0	0.2	0.44
Proteocephalid cysts	30.0	0	12.5	0	18.1	0	0	0	0	0	0	0	6.6	11.1
	0.6	0	0.68	0	0.18	0	0	0	0	0	0	0	0.06	0.22
Spirurid cysts	0	0	6.2	0	0	0	0	11.1	42.8	40.0	22.2	27.2	13.3	22.2
	0	0	0.06	0	0	0	0	0.11	1.1	1.0	0.66	0.9	0.13	0.77

drawing definite conclusions, for infestation may vary considerably in the same month of different years. In some cases a "moving average" has been used to straighten out a curve. Instead of plotting each per cent for the respective months, the average of three is used, which is plotted as follows: 1, 2, 3 are averaged and plotted at 2; 2, 3, 4 at 3; 3, 4, 5 at 4; etc.

PROTOZOA

A high percentage of infestation with intestinal protozoa usually remains fairly constant in different species of hosts throughout the year. In

TABLE 6. Seasonal Infestation of *Ambystoma opacum* (adults), Durham, N. C., October, 1934, to October, 1935. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Number hosts examined	14	6	9	2	11	9	14	6	11	10	7	11	9
Average length of hosts (m.m.)	79	99.9	108	113.5	97.5	97.1	99.5	90.8	91	91.3	89.5	84.8	110
Total % infest., intestinal Protozoa	92.8	50.0	66.6	50.0	90.9	100	78.5	66.6	100	100	100	36.3	77.7
Total % infest., adult Metazoa	92.8 27.5	100 43.8	100 35.6	100 6.0	100 162.0	88.8 14.1	100 37.0	100 18.8	90.9 17.3	100 16.4	100 10.7	100 44.4	100 20.6
Cryptobia	0	0	0	0	63.6	0	7.1	0	0	0	0	0	33.3
Cytamoeba	0	0	0	0	55.5	0	7.1	0	0	0	0	0	44.4
Eimeria	21.4	0	0	0	0	0	0	0	0	20.0	14.2	0	11.1
Eutrichomastix	14.2	0	0	0	0	44.4	0	16.6	0	30.0	28.5	0	11.1
Haptophrya michiganensis	7.1	0	11.1	0	9.09	11.1	14.2	16.6	0	0	0	0	22.2
Hexamastix	0	0	0	0	0	0	7.1	0	0	0	0	0	11.1
Hexamitus intestinalis	0	0	0	0	18.1	66.6	0	0	0	0	0	0	0
Prowazekella	7.1	0	0	0	35.5	11.1	57.1	50.0	72.7	60.0	42.8	27.2	55.5
Tritrichomonas	85.7	50.0	66.6	50.0	81.8	88.8	57.1	33.3	100	100	100	9.09	22.2
Brachycoelium	85.7 20.9	100 36.6	77.7 22.2	100 6.0	81.8 30.7	88.8 14.0	92.8 25.5	83.3 10.3	81.8 8.6	100 11.0	85.7 8.8	63.6 19.8	66.6 4.6
Capillaria inequalis	50.0 4.3	16.6 1.16	77.7 13.2	0 0	81.8 8.63	33.3 0.88	50.0 8.8	33.3 4.1	63.6 4.1	70.0 4.0	42.8 1.4	72.7 22.7	88.8 4.5
Cosmocercoides dukae	35.7 0.5	0 0	22.2 0.22	0 0	27.1 4.8	22.2 0.33	57.1 3.07	33.3 1.1	45.4 1.1	30.0 0.6	28.5 0.42	54.5 1.0	33.3 0.88
Diplostomulum ambystomae	85.7 235.07	66.6 205.1	88.8 111.3	0 0	63.6 142.6	44.4 209.0	64.2 65.5	50.0 58.6	72.7 77.6	80.0 207.5	85.7 189.7	81.8 274.5	66.6 140.1
Gorgoderina bilobata	0 0	0 0	0 0	0 0	0 0	0 0	14.2 0.28	50.0 3.1	54.5 3.3	30.0 0.8	0 0	27.2 0.9	33.3 0.88
Hannemania	0 0	66.6 6.0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	44.4 9.5
Plagitura	14.2 1.92	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Megalodiscus temperatus	0 0	0 0	0 0	0 0	0 0	0 0	7.1 0.7	0 0	0 0	0 0	0 0	0 0	0 0
Spirurid cysts	0 0	33.3 0.83	0 0	0 0	9.09 0.18	0 0	0 0	16.0 0.16	18.1 0.63	0 0	0 0	0 0	33.3 4.9

Ambystoma opacum, however, a definite increase occurs during spring and summer months (Fig. 9). This salamander breeds in fall whereas most other salamanders breed in spring. During spring and summer it lives in a terrestrial environment and so provides for reinfestation with intestinal protozoa. It migrates to water in fall where many flagellates may be lost. Other salamanders either maintain a more or less constant environment or migrate frequently from land to water, with the result that little variation in intestinal protozoa is exhibited.

TABLE 7. Seasonal Infestation of *Plethodon glutinosus*, Durham, N. C., October, 1934, to October, 1935. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	Oct.-Nov.	Dec.-Jan.	Feb.-Mar.	Apr.-May	June-July	Aug.-Sept.	Oct.
Number hosts examined	11	11	13	18	13	16	10
Average length of hosts (m.m.)	130.2	152.1	116.7	135.3	126.3	125.2	132.5
Total percent infest., intestinal Protozoa	100	100	100	77.7	76.8	93.7	100
Total percent infest., adult Metazoa	81.8 18.9	72.7 4.6	84.5 5.5	55.5 4.1	76.8 5.5	87.5 14.3	100 18.7
Cryptobia	0	27.2	23.1	0	0	0	0
Cytamoeba	0	0	7.6	5.5	0	0	0
Eutrichomastix	18.1	0	0	16.6	23.1	43.7	80.0
Haptophrya gigantea	0	0	7.6	11.1	0	0	0
Haptophrya michiganensis	9.09	0	0	0	15.3	12.5	40.0
Hexamastix	0	0	0	16.6	0	0	20.0
Hexamitus batrachorum	0	0	0	11.1	38.4	43.7	80.0
Hexamitus intestinalis	0	0	53.9	11.1	23.1	18.7	0
Karotomorpha swezi	0	0	0	16.6	0	18.7	0
Prowazekella	72.7	54.5	61.5	66.6	69.2	87.5	70.0
Tritrichomonas	90.9	100	38.4	50.0	0	0	0
Brachycoelium	54.5 3.4	63.6 4.4	69.2 3.7	33.3 2.6	53.8 3.8	81.2 9.3	80.0 10.5
Capillaria inequalis	0 0	0 0	0 0	30.7 0.5	23.07 0.46	31.2 0.68	60.0 2.5
Cosmocercoides dukae	18.1 0.35	36.3 0.36	38.4 1.7	38.8 2.1	23.1 0.3	18.7 0.3	30.0 0.7
Hannemania	54.5 14.9	0 0	7.6 0.07	0 0	15.3 1.0	31.2 4.0	50.0 5.7
Spirurid cysts	9.09 2.8	0 0	0 0	22.2 8.1	23.1 1.4	6.2 0.18	20.0 2.3

Tritrichomonas and Prowazekella are the commonest protozoan parasites and exhibit certain seasonal variations. Tritrichomonas tends to be more prevalent in winter months than during summer, while Prowazekella occurs more frequently in summer. Infestation with Tritrichomonas is by ingestion of active flagellates, while that with Prowazekella by ingestion of cysts (Wenyon, 1926). Hegner (1928) found that viable flagellates could be recovered from feces eight days later after being kept at temperatures vary-

ing from 5°-31°C. When feces are highly diluted with water, however, the osmotic pressure is so greatly changed that death occurs within a few hours. Cysts of various protozoa are viable for long periods of time outside of the host's body (Wenyon, 1926). During the increased migratory activity of salamanders in spring and summer, Tritrichomonas would be dropped in moist places and if not ingested within a short while would probably die. On the other hand, viable cysts of Prowazekella may be picked up

TABLE 8. Seasonal Infestation of *Triturus v. viridescens*, Lakeview, N. C., December, 1934, to November, 1935. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Number hosts examined	5	9	11	10	12	8	10	9	4	10	10	13
Ave. length of hosts (m.m.)	84.6	92.7	75.9	90.2	85.8	86.0	90.4	90.4	93.9	81.8	88.4	88.7
Total percent infest., intestinal Protozoa	100	100	100	90.0	100	100	100	100	100	80.0	100	100
Total percent infest., adult Metazoa	40.0 0.6	55.5 0.55	81.8 2.2	20.0 0.2	10.0 1.8	75.0 1.8	70.0 4.9	44.4 1.2	50.0 0.75	30.0 0.4	70.0 2.8	53.8 3.07
Cryptobia	0	0	100	40.0	100	0	0	0	0	40.0	0	0
Cytamoeba	0	0	9.0	0	0	12.5	50.0	33.3	25.0	20.0	0	0
Entamoeba	40.0	0	9.0	0	0	0	0	0	0	0	0	0
Eutrichomastix	80.0	0	0	20.0	0	75.0	30.0	44.4	50.0	0	30.0	38.4
Hexamastix	0	0	0	10.0	0	50.0	10.0	0	0	0	0	0
Hexamitus batrachorum	0	0	54.5	0	100	25.0	30.0	11.1	75.0	0	0	0
Hexamitus intestinalis	0	0	0	0	0	0	0	22.2	50.0	0	0	0
Karotomorpha swezi	0	0	0	12.5	0	0	50.0	0	0	0	0	0
Myxobolus	0	0	9.0	0	16.6	0	0	0	0	0	0	0
Prowazekella	40.0	66.6	63.6	90.0	83.3	12.5	80.0	44.4	100	0	50.0	61.5
Tritrichomonas	60.0	100	18.1	80.0	33.3	12.5	90.0	100	100	80.0	100	69.2
Brachycolium	0 0	0 0	9.0 9.09	10.0 0.3	8.3 0.83	12.5 0.87	30.0 2.0	22.2 0.55	25.0 0.25	0 0	30.0 1.5	38.4 1.7
Capillaria inequalis	20.0 0.4	55.5 0.55	72.7 2.18	20.0 0.2	8.3 0.91	62.5 1.0	70.0 2.9	33.3 55.5	25.0 0.5	30.0 0.4	60.0 1.3	30.7 1.3
Cosmocercoides dukae	20.0 0.2	0 0	0 0	10.0 0.1	8.3 0.08	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Proteocephalid cysts	0 0	0 0	18.1 0.18	10.0 0.1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Spirurid cysts	20.0 0.8	66.6 6.8	72.7 3.8	80.0 7.0	75.0 8.4	62.5 3.0	70.0 4.9	88.8 12.0	100 19.5	70.0 1.6	20.0 4.9	69.2 9.6

at any time during the migratory period. Maximum infestation with Karotomorpha is apparently correlated with winter, while that with Hexamastix and *Hexamitus intestinalis* with spring and summer. Infestation with these protozoa is similar to that with Tritrichomonas, through ingestion of active trophozoites. Hexamastix and Hexamitus have many closely-related free-living species. It may be that Karotomorpha is more adjusted to a parasitic existence and will perish if freed in water, whereas the more viable species, unrestricted by parasitic adaptations, will survive when deposited in this

medium. Seasonal variation would, therefore, tend to follow the activities of the host. The remaining intestinal protozoa appear too erratic for prediction of any periodic cycles. The presence of blood protozoa differs in various habitats, but in general, a low infestation occurs during June and July.

Total infestation with metazoan parasites also varies considerably with species of host. *Desmognathus f. fuscus* exhibits the most striking variations with respect to seasonal periodicity (Fig. 10). In this host a low percentage of infestation prevails from November to April and then increases rapidly to a maximum in September. It seems that hibernation during the cold months brings about an elimination of parasites; an increase apparently occurs during the summer migratory period. As a rule, greater infestation of salamanders with parasites occurs during warm months. High infestation is usually accompanied by a low average number of parasites per host.

TABLE 9. Seasonal Infestation of *Triturus v. viridescens*, Buchanan's Pond, Durham, N. C. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	July	October	December
Number hosts examined	13	19	12
Average length of hosts (m.m.)	90.4	88.0	95.2
Total percent infest., intestinal Protozoa	100	100	100
Total percent infest., adult Metazoa	100 9.0	95.0 7.5	91.6 4.5
Eutrichomastix	84.6	73.6	75.0
Hexamastix	30.7	26.3	0
Hexamitus batrachorum Hexamitus intestinalis	0 61.5	26.3 0	33.3 0
Prowazekella	53.8	94.7	91.6
Tritrichomonas	53.8	12.1	75.0
Camallanus	7.6 0.1	0 0	0 0
Capillaria inequalis	84.6 3.76	84.2 4.0	75.0 2.0
Cosmocercoides dukae	0 0	21.1 0.21	25.0 0.33
Plagitura	61.5 11.9	52.6 1.6	41.6 1.6
Megalodiscus temperatus	38.4 0.9	42.1 1.6	25.0 0.58
Spirurid cysts	92.3 22.5	26.3 3.1	33.3 1.7

TREMATODA

Brachycoelium is usually more abundant from May to September (Fig. 11) than during other months, particularly in *Desmognathus f. fuscus*. Although the life cycle of Brachycoelium is unknown, the infestation stage is probably in a food common to all salamanders. This food may be an in-

sect which is most abundant during April and May and infestation of the definitive host occurs after ingestion of the insect. Low infestation during winter indicates that this parasite is lost to a considerable extent during that period. Dead flukes have been found in the rectum in September and October.

The prevalence of Plagitura from April to July also indicates that the infestive stages of the life cycle is more abundant during this period. This trematode is closely correlated with aquatic habitats. It seems that hosts

TABLE 10. Seasonal Infestation of *Triturus v. viridescens*, Settling Pond, Durham, N. C. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	December	February	April
Number hosts examined	9	5	12
Average length of hosts (m.m.)	97.8	84.8	91.1
Total percent infest., intestinal Protozoa	88.8	100	100
Total percent infest., adult Metozoa	77.7 7.8	20.0 0.4	100 15.5
Cryptobia	0	100	91.6
Cytamoeba	0	0	25.0
Hexamitus batrachorum	0	60.0	0
Prowazekella	88.8	80.0	100
Tritrichomonas	77.7	60.0	8.3
Acanthocephalan cysts	11.1 0.3	0 0	0 0
Brachycoelium	11.1 0.1	0 0	16.6 1.0
Capillaria inequalis	77.7 2.7	20.0 0.2	83.3 6.5
Gorgoderina bilobata	0 0	0 0	16.6 0.2
Plagitura	33.3 1.6	20.0 0.2	58.3 5.0
Megalodiscus temperatus	11.1 1.0	0 0	41.6 2.9
Metacercariae	0 0	0 0	33.3 3.4
Spirurid cysts	33.3 6.4	40.0 0.2	16. 0.6

are infested through ingestion of contaminated foods (Stunkard, 1936). Cercariae, as a rule, migrate from intermediate hosts, snails, when water is warmed. This may account for spring and summer infestation of salamanders.

Amphistomes are erratic in seasonal distribution but tend to be present particularly from April to July. The cercaria of Megalodiscus penetrates and encysts in the *stratum corneum* of the host. This layer is shed more or less continuously, especially in spring. Infestation is accomplished by ingestion of molted skin. More cercariae are probably present in spring

TABLE 11. Seasonal Infestation of *Triturus v. viridescens*, Lake Powhatan (mountains), N. C. Upper Figure = Percentage of Infestation; Lower = Average Number of Parasites Per Host.

Parasites	March	June	October
Number hosts examined	28	24	32
Average length of hosts (m.m.)	97.9	92.2	97.9
Total percent infest., intestinal Protozoa	100	100	96.8
Total percent infest., adult Metazoa	92.8 9.7	79.1 2.9	65.6 2.8
Cryptobia	35.7	0	0
Cytamoeba	46.4	0	0
Entamoeba	0	0	3.2
Euglenamorpha	0	4.1	0
Eutrichomastix	0	12.5	59.3
Hexamastix	0	0	21.8
Hexamitus batrachorum	0	12.5	71.8
Hexamitus intestinalis	0	0	12.5
Nyctotherus cordiformis	0	0	6.2
Prowazekella	100	100	15.6
Brachycoelium	14.3 0.53	20.8 0.4	40.6 2.3
Capillaria inequalis	3.5 0.04	8.7 0.08	0
Plagitura	67.8 9.0	75.0 1.9	37.5 0.7
Oxyuris magnavulvaris	14.3 0.2	0	9.3 0.1
Spirurid cysts	25.0 0.4	58.7 3.1	21.8 0.5

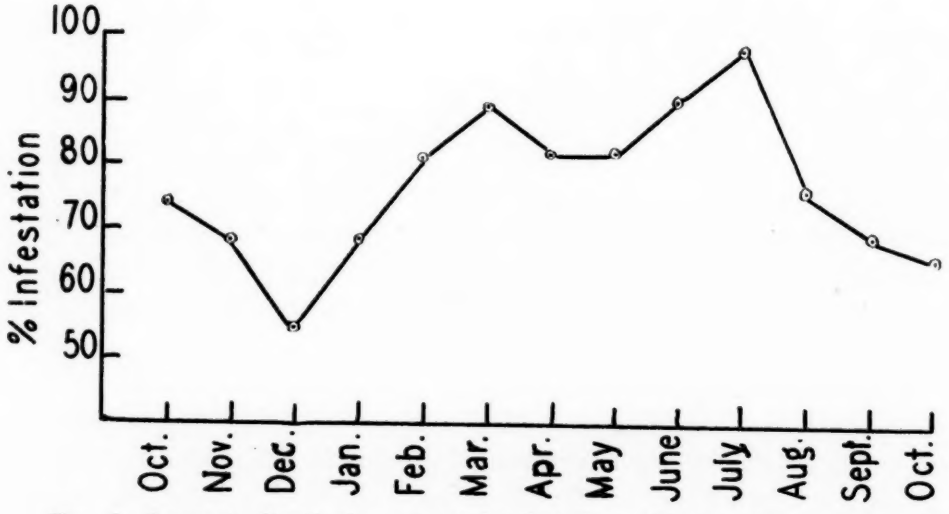

FIG. 9. Seasonal distribution of total infestation with intestinal protozoa in *Ambystoma opacum* (moving averages).

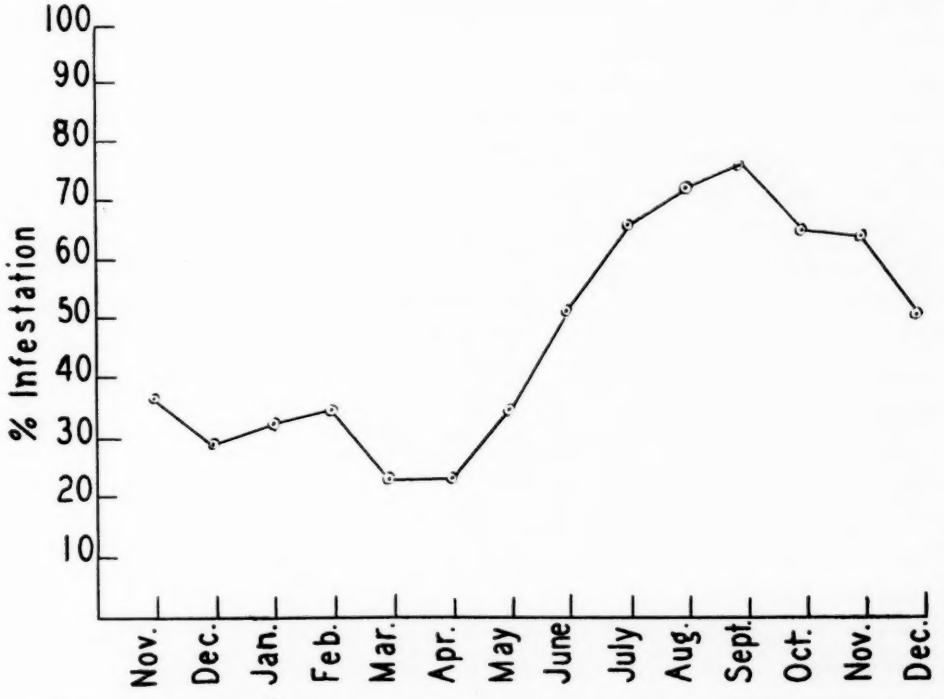

FIG. 10. Seasonal distribution of total infestation with metazoan parasites in *Desmognathus f. fuscus* (moving averages).

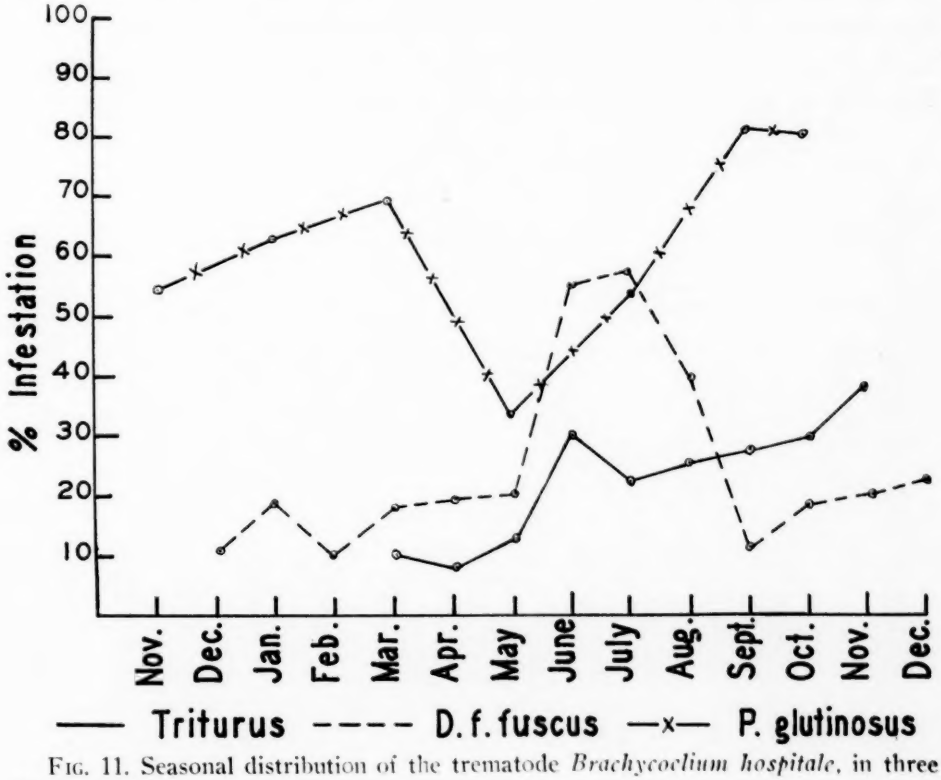

FIG. 11. Seasonal distribution of the trematode *Brachycoelium hospitale*, in three representative hosts.

during the breeding season. The majority of salamanders breed in water and so become infested at that time. Such encystment may account for erratic seasonal distribution of amphistomes. Cercariae penetrate the epidermis of the host, encyst, and are carried about for some time. When molting occurs, perhaps at some distance from water, infested skin may be eaten and intestinal infestation result.

Variations shown by bladder flukes are apparently correlated with a terrestro-aquatic environment. The life cycle of these trematodes requires two intermediate hosts, either two molluscs, or a mollusc and an aquatic insect larva (Krull, 1935). The prevalence of flukes from June to November may be due to greater activity and abundance of intermediate hosts while the water is warm.

As *Diplostomulum ambystomae* is a strigeid trematode, it probably develops from a furcocercous cercaria. Food habits of the adult salamander probably do not expose it to the cercaria. Nevertheless, the diplostomulum is more prevalent in adult hosts than in larvae, which indicates reinfestation after metamorphosis. In adults the incidence and average number of parasites per host exhibit a certain degree of periodicity. The diplostomula are most common from July to January and least common in spring (Fig. 12). The large number present in winter may be because the host, *Ambystoma opacum*, breeds in autumn, and is exposed to reinfestation at that time. The reduced number in spring indicates a failure of many of the parasites to live through winter. During the dry summer season parasites appear to be more abundant again, possibly because salamanders are exposed to infestation during the spring rainy season in consequence of swollen streams and

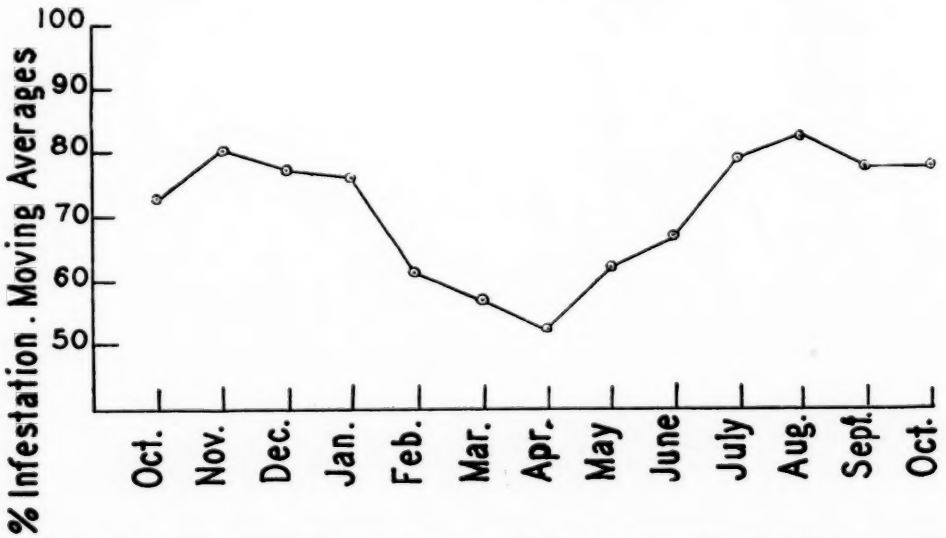

FIG. 12. Seasonal periodicity of infestation of *Ambystoma opacum* with the larval trematode *Diplostomulum ambystomae*.

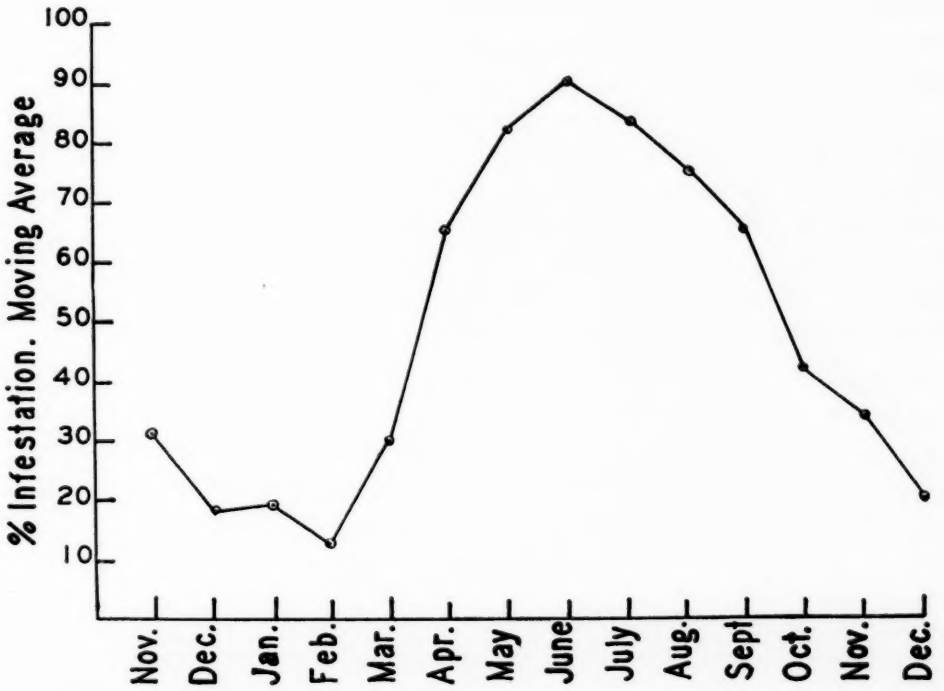

FIG. 13. Seasonal distribution of infestation of *Desmognathus f. fuscus* with encysted metacercariae.

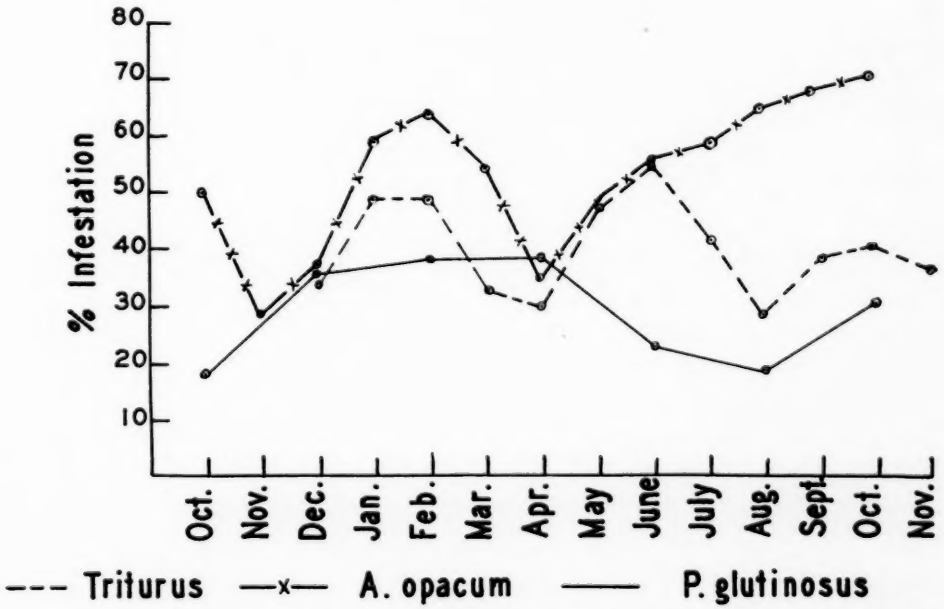

FIG. 14. Seasonal variation in infestation of salamanders with the nematode *Capillaria inequalis*.

flooded lowlands. Too few examinations for *D. desmognathi* were made to determine seasonal distribution. Results obtained, however, indicate the same variations as found for *D. ambystomae*.

Encysted metacercariae exhibit a pronounced seasonal periodicity in *Desmognathus f. fuscus* (Fig. 13), and to a certain extent in other hosts. Infestation is low from October to March and then rapidly increases to a maximum in June. Emergence of cercariae in spring and increased migratory activity of the host at this period probably brings about this seasonal variation. Failure to survive during winter may account for low percentage of infestation in this period.

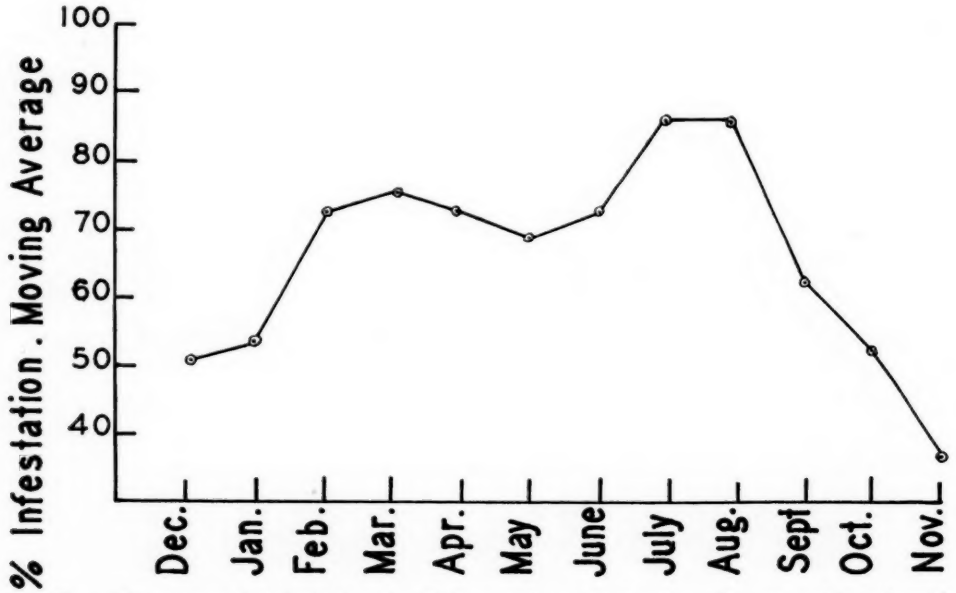

FIG. 15. Seasonal infestation in Triturus from Lakeview, N. C., with spirurid (nematode) cysts.

CESTODA

Adult cestodes are very erratic in distribution but tend to be more prevalent from June to November. Copepods are the known intermediate hosts for tapeworms of amphibia. The data suggest the prevalence of these crustacea during early summer. Cestode cysts are also erratic, appearing primarily from November to March.

NEMATODA

Capillaria inequalis is the most common adult nematode in the salamanders studied. A fairly well-defined seasonal variation is found in *Plethodon glutinosus* (Fig. 14). Infestation is high from December to May and low the remainder of the year. In other hosts, infestation appears to be low in April, and irregular during other months. *C. inequalis* is oviparous and this may account for its erratic distribution. Eggs are deposited in water

and are ingested with food. During dry seasons, the receding water may leave eggs to become carried about by any mechanical means present. Land hosts may then become infested by ingestion of eggs some distance from water. Fresh infestations seem to occur shortly after dry seasons.

Cosmocercoides dukae is more prevalent from April to October than during winter months, and reaches a maximum in May. Greater infestation in spring may be correlated with increased activity of the host after hibernation, during which time eggs of this nematode may become ingested.

Spirurid cysts reach a maximum infestation in July and August (Fig. 15). The low percentage reported from September to January may be correlated with decreased host activity. The rest of the nematodes found are quite erratic in distribution.

ACANTHOCEPHALA

Acanthocephalans are found almost exclusively during winter months. Infestation occurs after ingestion of an arthropod that probably is more abundant during the latter part of the summer.

ACARINA

The distribution of Hannemania indicates that infestation occurs in August and reaches a maximum in November. Very few individuals are found from February to July. Only the larval parasite infests salamanders. Apparently this mite is an adult during summer and eggs are laid in late summer. The newly-hatched larvae then infest salamanders and stay with them until the following spring.

HOST SPECIFICITY

Several parasites have been found in salamanders that exhibit a definite specificity for particular hosts. *Allocreadium pseudotritoni* is found only in species of Pseudotriton. This salamander is collected in the same habitats with Desmognathus and Eurycea. None of the latter hosts is infested with Allocreadium, which indicates a specificity for Pseudotriton rather than accidental parasitism. *Diplostomulum ambystomae* occurs only in species of Ambystoma, primarily in *A. opacum*, in the lowlands. *D. desmognathi* is present in species of Desmognathus in the mountains. In both cases other salamanders collected in the same habitats are subjected to similar conditions, yet remain uninfested. Members of the trematode family Gorgoderidae (bladder flukes) are more or less definitely limited to one or two host species. A predilection for specific hosts is indicated. On the other hand, some salamander parasites exhibit little or no host specificity.

MULTIPLE INFESTATION

Multiple infestation in salamanders examined by the writer is of common occurrence. One specimen of *Triturus v. viridescens* harbored four species of protozoa, two of trematoda, and two of nematoda. An *Ambystoma*

opacum was infested with seven species of protozoa, three of trematoda, two of nematoda, and one of cestoda. The data indicate that infestation with a varied parasitic fauna is closely correlated with a terrestro-aquatic habitat. Salamanders occupying such a region migrate frequently from water to land and may become infested with parasites that are peculiar to both aquatic and land regions.

It has been observed that in cases of pronounced infestation with a variety of parasite species, individuals are present in small numbers. When single species are present, however, large numbers often occur. The exclusive presence of one species of parasite may limit others. Salamanders with intestines filled with the ciliate Haptophyrya are usually free from metazoan parasites; if the latter occur, they are present in very small numbers. Brachycoelium in the intestine of such individuals has been found covered with Haptophrya which are attached to the trematode by means of their oral discs. Also, dead flukes have been found. The evidence indicates that heavy infestation with Haptophrya may bring about an elimination of some of the other parasites present.

It has been observed also, that the trematodes Brachycoelium, Plagitura, and Megalodiscus, when present in large numbers, are usually small, though mature. Crowding of many individuals within a small area may account for small size, for when these flukes occur in small numbers, they are much larger.

SIZE AND AGE OF HOST

Characteristic differences are found between infestations in larval and adult hosts (Tables 1-4). Larvae of Triturus from Buchanan's Pond are infested with but three species of protozoa as opposed to six for adults. One hundred per cent of adult individuals are infested, while only eight per cent of the larvae harbor protozoa. Plagitura is more abundant in larvae (88%) than in adults (52%). This indicates that this fluke infests young hosts and becomes eliminated as the latter increase in age and size. Young Triturus are infested with immature Plagitura, adults with mature. Bladder flukes are present in adult hosts only, whereas encysted metacercariae are limited to larvae. Infestation with adult nematodes is more common in adult (88.6%) than in larval salamanders (4%), largely because Capillaria is absent from larvae but infests 81.5% of adults. Four species of nematodes are found in adult, only one in larval hosts.

Desmognathus f. fuscus from Settling Pond exhibit similar differences in infestation. 36.3% of adult individuals harbor blood protozoa, as opposed to 13.6% of larvae. Cytamoeba is not found in larvae. Intestinal protozoa are found in 99.3% of adults and in 77.2% of larvae. Seven species of protozoa occur in mature hosts, five in larval. Adult salamanders are infested with five species of trematodes, the larvae with but one. Metacercariae

are present in adults but absent from larvae. Mature tapeworms are found only in adult hosts, while encysted cestodes are more prevalent in larvae. Nematodes and acanthocephala are more abundant in adults.

Larvae of *Ambystoma opacum* are infested with 31.2% of blood protozoa, adults with 13.4%. Cryptobia is common in larvae, whereas Cytamoeba is found only in adults. Mature hosts harbor 80.6% of intestinal protozoa, larvae but 37.5%. Intestinal and bladder flukes, nematodes, and mites occur only in adults, while acanthocephalans are present usually in larvae.

The data indicate that percentage of infestation and average number of parasites per host increase significantly with an increase in size and age of hosts.

RARE OR ACCIDENTAL PARASITES

Nyctotherus cordiformis, Eimeria ranarum, and *Entamoeba ranarum* are common parasites of frogs. *Euglenamorpha hegneri* is usually found in frog tadpoles. *Megalodiscus intermedius* infests frogs. *Myxobolus conspicuus* commonly occurs in fresh-water fishes. *Pomphorhynchus bulbicolli* has been reported only from fish hosts. All of these parasites have been observed in isolated individual salamanders and in very small numbers. They may be considered as rare or accidental parasites.

DISCUSSION AND CONCLUSIONS

Though amphibian parasites from North America have been extensively studied since the opening of the present century, no intensive ecological surveys had been attempted until the work of Brandt (1936) on frogs. By far the majority of parasites reported for Amphibia have been those of Salientia. Brandt's check list indicates that parasites have been found associated with eighty-two species and sub-species of frogs. The total number of salientian species inhabiting North America is between ninety and one hundred. Stejneger and Barbour (1933) recognize eighty-six species and sub-species of salamanders from this continent. From only thirty-five of these have parasites been reported. It is apparent that frogs and toads have been extensively studied and that salamanders have been studied only superficially. The reasons are quite apparent. Salientians are larger, more easily observed, ubiquitous, and frequent fairly open habitats. They have long had an important place in the teaching of zoology. Economically, they are perhaps considerably more important than their salamander relatives. Salamanders, on the other hand, are very retiring. During the day they seek hiding places under rocks, logs, and other débris. Rarely are they found in open places. Very few are used commercially.

The only extensive ecological study of North American amphibian parasites is that of Brandt (1936) on those of North Carolina Salientia. Mann (1932) made a preliminary survey of North Carolina salamander parasites

but has published nothing. Holl (1932) included the newt, *Triturus v. viridescens*, in a study of the helminth parasites of some fishes and amphibians. The ecology of Japanese salamanders was studied by Pearse (1932). Scattered ecological notes throughout the literature refer to particular amphibian parasites. Other than these, no extended survey of the relationships between Amphibia and parasites has been attempted.

It seems to the writer that such surveys on salamander parasites as are presented in this paper should perhaps prove of more interest from a scientific point of view and of more fundamental value than those on the parasites of salientians. Salamanders are recognized as more primitive of the two groups. More of the parasites infesting salamanders are found in fishes than are those of frogs and toads. Parasites of salamanders, salientians, and reptiles overlap to a considerable extent. Relationships between piscine, amphibian, and reptile parasites should be more easily demonstrated from a study of salamander than of salientian parasites. Perhaps salamanders may act as reservoirs for some fish parasites in the absence of fishes, and in their migrations infest the fish fauna of new drainage systems. Infestation with various species of parasites in a given habitat is, in general, much less in salamanders than in frogs and toads. More definite conclusions as to the relation of parasites to habitat, seasonal periodicity, and other phenomena are obtained from this condition, than from one where numerous parasite species are encountered.

Total infestation of salamanders with parasites tends to range from high in aquatic, erratic in terrestro-aquatic, to low in terrestrial habitats. Pearse (1920) concludes that fishes with a most restricted habitat have a higher number of individuals infested, but carry few species of parasites and have a small average number of parasites per host, whereas (1924a) those with a variety of habitats are correlated with a large number of species of parasites as well as with a large percentage of infestation. Little (1928) found aquatic more heavily infested than land salamanders. Rumbold (1928) and Brandt (1936), dealing with turtles and salientians respectively, came to this same general conclusion. On the other hand, Mann (1932) and Pearse (1932) found that terrestrial species of salamanders have about as many parasites as aquatic species. Aquatic salamanders are usually restricted more or less to one specific habitat and would be expected to have a larger number of individuals infested with the parasites present. Terrestrial salamanders, however, though also restricted to a single habitat, are in one in which the number and kind of parasites are usually low. The terrestro-aquatic hosts migrate frequently, have a variety of habitats, and consequently harbor numerous species of parasites.

Twenty-eight species of protozoa have been recorded from North American salamanders, five of which may be considered as accidental. Blood protozoa are primarily present in aquatic hosts at Durham, North Carolina, er-

ratic in others. In the mountains of North Carolina terrestro-aquatic hosts are more heavily infested. Brandt (1936) found greater infestation in aquatic Salientia than in others. Leeches are the known intermediate hosts for certain of these parasites (Noller, 1913, 1913a; Wenyon, 1926; Hegner, 1929) and occur almost exclusively in an aquatic environment.

Intestinal protozoa are commonly found in all species of salamander hosts. Few species occur in aquatic, many in terrestro-aquatic and terrestrial hosts. The percentage of infestation tends to be greatest in terrestro-aquatic salamanders. It appears that these hosts obtain their varied infestations by ingesting contaminated food in their frequent migrations from water to land. Hegner (1929) found that newts (Triturus) retain their intestinal flagellates but lose their trypanosomes when they change from an aquatic to a terrestrial habitat. He concludes that under terrestrial conditions reinfestation with intestinal flagellates probably occurs frequently by ingestion of contaminated food, but that no reinfestation with trypanosomes occurs on land because of the absence of leeches in this habitat. Mann (1932) observed that *Hexamitus batrachorum* and *Haptophrya michiganensis* were limited to aquatic and terrestrial habitats respectively. Brandt (1936) found frogs of terrestrial habits more heavily infested with ciliates than were other hosts. In a study of the method by which *Hemidactylium scutatum* (Schlegel) becomes infested with Haptophrya, Woodhead and Kruidenier (1936) found that the salamander is attracted by the motion of the protozoa and swallows them. They believe that this is the only method of infestation. The breeding habits of the host would promote this type of infestation. The females remain on the nest until after the eggs are hatched. The ciliates are shed in the feces in a non-cystic normal condition. The young eat these and thus become infested. This infestation is carried through metamorphosis into the adult. The present writer has found terrestrial hosts frequently parasitized with this ciliate which indicates that water is not absolutely essential for its transmission.

In some localities there may be a tendency for Protozoa to occur in a progressive series with respect to habitat preference. Mann (1932) found *Desmognathus f. fuscus* more infested with trichomonads than was Triturus; *Plethodon glutinosus* more with Prowazekella than was Desmognathus, and that trichomonads were absent from the former. Such a series was observed to a certain extent by the present writer, but an examination of many more hosts indicated that much overlapping of species occurs. Pearse (1932) found no significant differences between the intestinal flagellates of aquatic and terrestrial salamanders in Japan. Little difference is exhibited with respect to protozoan infestation in mountain and lowland salamanders.

All of the twenty-five endoparasitic trematodes reported from North American salamanders have been found primarily in aquatic or terrestro-aquatic hosts. The present study indicates that hosts living in a terrestro-

aquatic environment are parasitized with more species of trematodes than those hosts living in other types of habitats. Rumbold (1928) found that trematodes of turtles tend to increase steadily from land to aquatic habitats. Mann (1932) observed that aquatic and terrestro-*aquatic* salamanders have the greatest variety of trematode species, and that *terrestro*-aquatic and terrestrial hosts harbor more trematodes per individual than do other hosts. Pearse (1932) concludes that salamander flukes are confined largely to aquatic habitats. More trematode parasites were found in aquatic than in terrestrial Salientia by Brandt (1936).

Many trematodes tend to be found in few species of salamanders. The writer's check list indicates that eighteen species are found in only one species of host. Of these at least seven have been also found in either reptilian or piscine hosts. A majority of the eighteen species belong to genera of which species have been reported from other hosts, mostly fishes. Many trematodes probably originally infested fishes and secondarily have taken up an abode in salamanders. *Brachycoelium hospitale* is the only trematode which has been found common to a majority of salamanders in all habitats examined. This is the only trematode that Rumbold (1928) found in land turtles. The present study indicates its general prevalence in terrestrial hosts, though Brandt (1936) correlated it with aquatic habitats. Seventeen species of hosts harbor this fluke. Undoubtedly it will be found in many salamander species not yet examined. It has been observed in frogs and reptiles, but as far as the writer is aware, never in fishes. Trematodes, therefore, appear to indicate the relationship of salamanders to fishes on the one hand, and to salientians and reptiles on the other.

Salamanders in the lowlands contain a more varied trematode fauna than do those in the mountains. This fact agrees with the observations of Pearse (1932). The particular invertebrate fauna serving as intermediate hosts for flukes are apparently more abundant in the lowlands. In an analysis of the host-parasite-habitat relationship of the trematode type which involves one species of vertebrate host, one species of trematode, and one species of snail, three distribution areas, the range of each organism, must be considered. Usually the ranges of intermediate and definitive hosts do not coincide exactly. The range of the parasite, therefore, is more or less limited to areas where hosts overlap.

Larval flukes are primarily associated with an aquatic or terrestro-*aquatic* habitat. This correlation is in agreement with the known methods of entrance of cercariae by active penetration. Mann (1932) found diplostomula limited to such habitats, as Brandt (1936) did encysted metacercariae. There is apparently no definite limitation to any particular region, as both encysted and excysted species have been observed in the mountains and lowlands. According to Van Cleave and Mueller (1934) the habits of the final hosts, which are probably birds, would agree with observed facts, for

they are ubiquitous over the various regions and drop their egg-bearing excreta everywhere.

The occurrence of tapeworms is essentially correlated with an aquatic environment. Adults are never found outside of a host's digestive tract. Habitat relations, therefore, are much more restricted than for trematodes. Copepods are known to serve as intermediate hosts for salamander cestodes (Thomas, 1931). These crustaceans abound in the various aquatic areas studied. Seven species of adult cestodes have been found in North American salamanders, only one of which has been observed in the present study. Five species are found in individual host species, whereas immature forms occur in many hosts. It seems that larval salamanders eat copepods and that adults do not, or do rarely. Many larvae, therefore, become accidentally infested but the cestodes mature in few salamanders species. Adult tapeworms and plerocercoids are fairly equally distributed in the mountains and lowlands.

Nematodes are probably the commonest parasites of salamanders; thirty-six species have been reported from North American hosts. Many are specific either for certain hosts or for particular localities, so that distribution studies indicate little difference in total infestation with respect to habitat. Although the present study indicates that aquatic salamanders in Durham are the most heavily infested, it is probably because of the prevalence of Capillaria in aquatic regions. *Oxyuris magnavulvaris* predominates in terrestro-aquatic hosts in the mountains and these salamanders show the highest infestation there. Rumbold (1928) found aquatic turtles the least parasitized with nematodes, terrestrial most. Mann (1932) observed that the *terrestro*-aquatic salamander, *Ambystoma opacum*, had the greatest variety of these worms. The nematodes of Japanese salamanders are erratic in distribution (Pearse, 1932). *Cosmocercoides dukae* is widely distributed among salamanders of the lowlands and the writer's check list indicates that it is the commonest nematode of all. It is also commonly found in salientians and reptiles (Harwood, 1932) but not in fishes. Oxyuris is also widespread, occurring in ten species of salamanders in the mountains. On the whole, however, the majority of nematodes are found in few host species. The life cycles of various nematodes peculiarly adapt the worms for particular environments. Oviparous species tend to be more common in terrestrial habitats than in aquatic, whereas viviparous species predominate in aquatic regions. In general, lowland salamanders are more infested than mountain hosts with nematodes.

Acanthocephala are rare parasites of salamanders; only three species have been reported from North America. These worms are essentially correlated with aquatic habitats. Pearse (1932) found this true for Japanese salamanders. Acanthocephalans are generally associated with a host in all stages of their development (Van Cleave, 1919a) so that, as Pearse

suggests, they are probably confined in salamanders wholly to aquatic habitats by their dependence on particular intermediate hosts. These worms are equally abundant in mountain and lowland habitats.

The older the habitat, the more parasite species may be expected to occur. Rumbold (1928) found that with an increase in the age of a habitat there is an increase in the number of trematode species, but that there is little influence on the nematode fauna. Holl (1932) also found that the number of parasites increased with the age of a lake. In a study of an artificial lake, Hunter (1932) found an unusually low infestation with fish parasites. He concluded, however, that as the lake grows older, with the concomitant increase in snail fauna and macroscopic water plants for snail food and protection, the number of parasites will also increase. Limited areas may yield high or low percentages of infestation according to the number of parasite species present. Fasten (1922) observed that restricted areas provide for the rapid spread of parasitic infestation. Pratt (1919) states that small inclosed bodies of water may contain more parasites than those of larger size because the fishes cannot escape by migration. Pearse (1924a), however, considers that the "size of a lake does not appear to be correlated with the degree of parasitic infection of its fishes."

Seasonal periodicity is exhibited by many of the parasites found in this study. Pearse (1924a) found that perch have most parasites in spring. Hausmann (1897) thought that perch had few parasites when little food was eaten in cold weather, but Pearse did not find this correlation. Probably most seasonal variation can be correlated with the life cycles of the parasite and various hosts, and with seasonal and physiographic changes. Many parasites do not seem to show any periodicity, yet such phenomena may occur and generations may overlap in the same host. A few mature Brachycoelium and Plagitura have been found with numerous immature individuals, which indicates reinfestation. As Van Cleave (1916) says, "the mere fact that a parasite is present in a definitive host for the entire year is no proof that there is no periodicity."

Little seasonal variation is found in the various protozoan parasites encountered. In a few instances individual species do tend to exhibit periodic changes. In Japanese newts, Pearse (1932) found lowest infestation in August. Flagellates like Tritrichomonas and Karotomorpha, more or less well adjusted to a parasitic existence, easily perish on being deposited in water (Hegner, 1928), but will survive in feces dropped on the ground. Flagellates like Hexamastix and Hexamitus, still primitive with respect to parasitism, may be safely deposited in water. On the other hand, cysts of some Protozoa, like Prowazekella, are usually extremely viable whether deposited on land or in water. The correlation of the life cycle of the parasite with increased activity of the host in spring will bring about a decrease

in infestation with some Protozoa and an increase in others. The decreased activity during dry months and in fall would tend to reverse this situation.

Total infestation with metazoan parasites does exhibit certain seasonal peculiarities. As a rule, greater infestation of salamanders occurs from April to September than during colder months. Most salamanders hibernate during winter. Animals kept in captivity tend to lose their parasites (Nicoll, 1914). It should be expected, therefore, that hibernation would also bring about an elimination of parasites in that there is little or no chance for reinfestation. Blanchard (1903), studying hibernating marmots, observed no intestinal worms during hibernation. Ward (1909), on the other hand, found that intestinal parasites of frogs are retained during hibernation, reaching a maximum infestation immediately after spawning in spring. Brandt's (1936) findings agree with those of Ward. Triturus has a maximum infestation during winter, according to Holl (1932). Rumbold (1928), however, observed that the trematode, *Heronimus cheyledrae* MacCallum, 1902, has a lowest percentage of infestation during hibernation. The data from the present study indicate that infestation is correlated with the period of reproduction when the majority of salamanders migrate to water. This period is usually in spring. Trematodes follow this general trend more closely than do other parasites. Cerceriae become active in spring and migrate from their snail hosts (Cort, 1922; Miller and Northup, 1926; McCoy, 1928; Rees, 1931). Infestation with larval flukes in spring would bring about maximum parasitism with adult individuals in summer and fall. The breeding of *Ambystoma opacum* in fall provides for infestation with diplostomula. The amount of rainfall may also be correlated with the abundance of some parasites. The amount and time of rainfall, according to Fortner (1923), affects the presence of parasites in a given locality in any one year.

Cestodes are more prevalent from June to November and thus appear after the spring breeding season. Brandt (1936) found this true for frog tapeworms. Though studying a limited amount of material, Pearse (1921) observed that Necturus examined in November was infested with Crepidobothrium, but that an individual examined in May had none.

Infestation with nematodes follows this same general trend though individual species indicate specific variations. Rumbold (1928) found that nematodes as a whole are few before hibernation, increase in spring months, and then decrease in late summer and autumn. Lung nematodes of Japanese salamanders are most abundant in spring (Pearse, 1932) and nematode cysts in winter. The writer finds, however, that nematode cysts are most abundant in July and August in North Carolina salamanders.

Acanthocephalans are found almost exclusively during winter months. Pearse (1932) found this true for these parasites in Japanese salamanders, but earlier (1924a) had found perch in Wisconsin lakes more heavily in-

fested in spring. Apparently these worms do best at low temperatures. The necessity of a host at all stages of development may also account for their prevalence in winter. Eggs are shed in the water when hosts migrate there to breed. Ingested by proper intermediate hosts, the eggs hatch and the resulting larvae remain until these hosts are eaten by either a second intermediate or a definitive host later in summer. Mature worms would, therefore, appear during winter.

The following list gives a general idea of conditions found by the writer with respect to seasonal distribution of parasites. Each type is given in connection with the particular season when it occurs in maximum numbers:

Spring: Blood protozoa, Capillaria, Cosmocercoides, Hexamastix, *Hexamitus intestinalis.*

Summer: Amphistomes, Brachycoelium, encysted metacercariae, Gorgoderidae, Hexamastix, Plagitura, Prowazekella.

Autumn: Allocreadium, adult cestodes, diplostomula, nematode cysts, Omeia, Oxyuris.

Winter: Acanthocephala, blood protozoa, Capillaria, cestode cysts, Hannemania, Karotomorpha, Tritrichomonas.

All seasons, or erratic: Camallanus, Oswaldocruzia, Physaloptera, most intestinal protozoa.

Some organisms are so well established as parasites that they occur in their particular host with a high incidence (Allocreadium, Diplostomulum) and frequently in great numbers in a single infestation *(Diplostomulum ambystomae).* Some are evidently able to infest and grow to maturity in a number of different hosts (Plagitura, Gorgoderidae) but in such cases a particular host species is usually found to be the most favorable one, as shown by greater frequency and intensity of infestation. Such a particular host may be considered as the natural host of the parasite, which passes only incidentally to other hosts capable of supporting it. From selected examples of host-specificity among protozoans, worms, and arthropods, Becker (1933) concludes that a particular parasite will develop normally in as many hosts as provide for it adequate environmental conditions and mode of entrance, and that a particular host may harbor few or many parasites regardless of taxonomic relationships, according to the milieu and opportunities for entrance it provides. Linton (1910) concluded that the restriction of adult stages of helminths to a few closely related species of hosts has a probable explanation in the physiology of the digestive processes rather than in food habits of hosts. The parasitic faunas of closely related salamanders are usually much alike, even when several hosts are found in dissimilar geographic ranges. Van Cleave (1919a) found strong evidence for host-specificity in fresh-water Acanthocephala. The specificity of Strongyloides species for their hosts is regarded by Sandground (1925) as the best means of determining the specific standing of the parasites. Rum-

bold (1928) observed that Camallanus and oxyuroids (Nematoda) are fairly specific for certain organs. These worms are intermingled in the host's intestine after hibernation, but two or three months later are segregated into definite zones. The writer has found that Cosmocercoides, Oxyuris, and Omeia are prevalent in the rectum of salamanders, Capillaria in the whole intestine, Camallanus in the small intestine and Physaloptera in the stomach. Canavan (1931) finds this same general trend, *i.e.*, *Physaloptera turgida* Rudolphi, 1819 and *Cruzia tentaculata* Rudolphi, 1819 in opossums, and *Trichuris ovis* (Abildgaard, 1795) and *Haemonchus contortus* Rudolphi, 1803 in ruminants, each taking up a different location in the intestine.

Pearse (1932) believes that host-specificity is probably as important a factor in the occurrence of salamander parasites in Japan as general habitat. Brandt (1936) found little or no evidence of a strict host-specificity in frog parasites. Ingles (1936), however, finds marked specificity among frog trematodes while the nematodes exhibit it very much less. The present study indicates this same specificity, particularly among the Gorgoderidae, as Ingles suggests. Ingles found no trematodes in the salamanders from California that he examined, *Batrachoseps a. attenuatus* (Eschscholtz), *Aneides fereus* Cope, *A. l. lubugris* (Hallowell), and *Ambystoma californiensis* Gray. This is somewhat difficult to understand, as he notes, for at least one trematode, Brachycoelium, has been found in many salamanders in North America and in other Amphibia from California. The study of few specimens of but four species of hosts, however, may account for the absence of Brachycoelium and other trematodes.

Salamanders of the terrestro-aquatic type have a large variety of parasitic fauna. Pearse (1924a) found that lakes with the widest range of territory and opportunity for fishes to invade the greatest variety of habitats have the highest average infestation per fish. In any given host, according to Ward (1918), only a few parasites may be found, or on the other hand, the number of individuals and species of parasitic worms in a single host may be very large. Brandt (1936) found multiple infestation commonly occurring in frogs.

Whenever multiple infestation occurs, individual species of parasites are usually represented by small numbers. When single species are present, however, large numbers prevail. There probably is a tendency of some parasites to limit either the presence or abundance of others. Van Cleave (1918) has never observed the occurrence of two different species of Acanthocephala within the same host individual. Stunkard (1932), studying the cestode Cittotaenia in hares and rabbits, believes that the presence of one species in the adult stage prevents infestation by another in the same genus. Cross (1934) found that two parasites, Neoechinorhynchus and Proteocephalus, are mutually limiting when found in large numbers in one host. Payne (1923) showed that infestation with hookworm tends to drive

out Ascaris in dogs. When a species of parasite is represented by many individuals, the size is usually small, whereas these parasites are much larger when present in few numbers. Crowding within a small area may account for small size.

Difference in degree of infestation within the same salamander species is frequently influenced by the age of the host. Definite correlations between an increase in size and age of individuals and an increase in amount of infestation has been observed in *Triturus v. viridescens, Desmognathus f. fuscus,* and *Ambystoma opacum.* Change in food habits and in some case, habitat, along with physiological changes in metamorphosis from larva to adult, probably account to a large extent for observed differences. Van Cleave (1919a) frequently observed that young and very small fishes may be free from acanthocephalan infestation, even though larger and presumably older specimens of the same species regularly carry parasites. Laboratory rats have been demonstrated to exhibit age resistance against the nematode *Nippostrongylus muris* (Yokogawa, 1920) by Africa (1931). Pearse (1932) and Brandt (1936) found significant correlations between body size and parasitic infestation. A decrease of protozoan species but an increase of metazoan, with an increase in age of tadpoles was observed by Chandler (1936).

Most salamander parasites do little injury to their hosts. Larval Hannemania in the skin, however, produce sores and exudates. Sometimes one or more legs are swollen with these mites. Definite histolytic action is apparent. A mechanical irritation is exhibited and possibly the sores serve as foci for bacterial infection. Some specimens of *Ambystoma opacum,* highly infested with *Diplostomulum ambystomae* in the body cavity, were swollen and sluggish in movement. The coelomic fluid of the host was milky in appearance. The intestines of many diplostomula contained erythrocytes. Some injury, therefore, was experienced by the hosts. Although the whole intestine of some hosts have been found swollen with thousands of Haptophrya, no external symptoms were observed. Van Cleave (1919a) found that Acanthocephala may inflict injury upon a host either mechanically or physiologically through interference with the normal functioning of organs. Pratt (1919) states that parasites may cause disastrous epidemics in which thousands of fishes die, but Pearse (1924a) found that most fish parasites do little harm to their hosts. Gyrodactylus has been found as a menace to fish hatcheries causing "fin-disease" (Van Cleave, 1921). Degenerative changes in the ovaries of fishes, producing sterility, by tapeworm larvae have been observed by Hunter (1928). Nigrelli (1929) found that trypanosome-free newts weighed almost twice as much as infested newts. The most injurious parasites of North Carolina salamanders appear to be mites, Acanthocephala, and diplostomula.

In an ecological study of this nature the ideal situation would be to know the entire life cycle of every parasite encountered. Factors influencing seasonal variations and host and habitat relationships could then be much more accurately determined. Often life cycles are assumed because the known stages of one parasite resemble those of another of which the whole cycle is known. Actually, the unknown stages of the former may differ considerably from the corresponding stages of the latter.

SUMMARY

1. The ecology of the parasites of about a thousand salamanders of North Carolina, representing nineteen species, has been investigated. It is believed that a study of salamanders with respect to the ecological aspect of their parasites will yield more comprehensive conclusions as to relationships between the phyla Pisces, Amphibia, and Reptilia, than will similar studies on Salientia.

2. Brief notes on the ecology of the various hosts studied are presented. The salamanders are from habitats which range from aquatic to terrestrial. Hosts were collected at five localities in the Piedmont area near Durham and at several points in the mountains around Asheville, North Carolina.

3. Exclusive of encysted larvae, parasites were found as follows: Protozoa, 16; Trematoda, 10; Nematoda, 8; Cestoda, 2; Acanthocephala, 1; Acarina, 1.

4. Percentage of infestation and average numbers of parasites per host are more or less closely correlated with habitat. Total infestation tends to range from high in aquatic, erratic in terrestro-aquatic, to low in terrestrial areas.

5. Terrestro-aquatic salamanders have a variety of habitats and, consequently, harbor more species of parasites than do others.

6. In some localities there may be a succession of parasitic protozoan types in salamanders from different habitats; in general, blood Protozoa are limited to aquatic habitats, intestinal Protozoa to terrestrial; both overlap in the terrestro-aquatic areas.

7. The older the habitat, the more parasite species there may be expected to occur.

8. In general, lowland salamanders are more heavily infested than are those in the mountains.

9. Greater infestation occurs primarily during summer rather than during other seasons.

10. A considerable degree of host-specificity occurs in many parasites observed.

11. Multiplicity of infestation is of common occurrence.

12. There is probably a tendency for some parasites to limit either the presence or abundance of others.

13. An increase in age and size of the host is associated with an increase in infestation.

14. Few salamander parasites injure their hosts. The most harmful types are probably mites, Acanthocephala, and Diplostomula.

15. A list of parasites of North American salamanders and a host list are presented in Appendix 1.

16. Keys to parasites of North American salamanders, arranged under appropriate groups, are presented in Appendix 2.

APPENDIX 1: PARASITE AND HOST LISTS

It is most convenient for workers in parasitology to have at their disposal check lists of parasites of different animal groups. Far too few such catalogues are available. It is hoped that the condensation of all available literature on salamander parasites into the following check lists will be an aid to future workers in this field.

PARASITES

This check list includes every parasite that has been reported from North American salamander hosts, as far as can be determined. The complete classification of each parasite, with correct scientific names, is given. Synonymy has been reduced to the presently recognized name wherever possible. It has been considered desirable to state briefly the reasons for such synonymy in some cases. The names of hosts, given completely in the host list (p. 000), are abbreviated here by omission of authors' names and dates. References to bibliography are given by author's name, date, and page number where possible. An asterisk in front of a parasite and host indicates that a specimen has been encountered in the present study. Hosts without reference indicate new host records. There is no universal agreement among parasitologists as to the exact status of many of the major groups that are used in systematic classification. The classification of the Protozoa is based on Wenyon (1926), of Trematoda on Kukenthal and Kumbach (1928) and Poche (1925), of Cestoda on Poche, of Nematoda on Yorke and Maplestone (1926), of Acanthocephala on Van Cleave (1919a, 1931), and of Acarina on Ewing (1929).

PROTOZOA

Sub-Phylum: Plasmodroma
Class: Sarcodina Hertwig and Lesser, 1874
Sub-Class: Rhizopoda v. Siebold, 1845
Order: Amoebida Claparede and Lackmann, 1858
Family: Amoebidae Bronn, 1859

**Entamoeba ranarum* Grassi, 1879. Rectum.

Host: **Triturus v. viridescens.*

An amoeba seen in the rectum of European salamanders (Chatton, 1910 and Alexieff, 1912, may possibly be this species (Wenyon, 1926: 233).

Class: Mastigophora Diesing, 1865
Sub-Class: Phytomastigophora Calkins, 1909
Order: Euglenoidida Blochmann, 1895
Family: Euglenidae Stein

**Euglenamorpha hegneri* Wenrich, 1923. Rectum.

Host: **Ambystoma maculatum, *Triturus v. viridescens.*

Sub-Class: Zoomastigophora Calkins, 1909
Order: Protomonadida (Blochmann, 1895)
Sub-Order: Eumonadia
Family: Trypanosomidae Doflein, 1901

Trypanosoma sp. Blood.

Host: *Triturus v. viridescens.* Laveran and Mesnil, 1912: 859; Castellani and Chalmers, 1913. North America.

Trypanosoma diemyctyli Tobey, 1906. Blood.

Host: *Triturus v. viridescens.* Tobey, 1906: 132, 1906a: 147; Ogawa, 1913: 268; Hegner, 1921: 105, 1929: 56; Nigrelli, 1929b. North America. (*Karotomorpha*, new name.)

Travis (1934) calls attention to the fact that *Tetramastix* was first used for a rotifer. Therefore he suggests *Karotomorpha*. He finds that Tetramastix, Polymastix, Tetramitus, and some species of Monocercomonas are synonymous and should be placed under this new generic name.

**Karotomorpha swezi* (Grassi, 1926) Travis, 1934. Rectum.

Syn.: Tetramitus Perty, 1852.
Polymastix bufonis Swezy, 1916; Tanabe, 1926.
Tetramastix swezi Grassi, 1926.

Host: *Ambystoma tigrinum.* Travis, 1934: 280. California.
Batrachoseps a. attenuatus. Ibid.: 279.
Eurycea longicauda. Ibid.; Tanabe, 1926: 97.
Triturus torosus. Swezy, 1916: 136; Travis, 1934: 279.
**Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Plethodon cinereus, *P. glutinosus, *P. metcalfi, *P. yonahlossee, *Pseudotriton r. ruber, *Triturus v. viridescens.*

Karotomorpha bufonis (Dobell, 1908) Travis, 1934. Rectum.

Syn.: *Monocercomonas bufonis* Dobell, 1908; Alexieff, 1911.

Host: *Ambystoma tigrinum.* Travis, 1934: 281. California.

Karotomorpha ambystomae (Das Gupta, 1935). Intestine.

Syn.: *Tetramastix ambystomae* Das Gupta, 1935.

Host: Ambystoma sp. Das Gupta, 1935: 223. Maryland.

Monocercomonas melolonthae Swezy, 1916. Intestine.

Host: *Ensatina eschscholtzii*. Swezy, 1916: 136. California.

Family: Cryptobiidae Poche, 1913

**Cryptobia borreli* (Laveran and Mesnil, 1901). Blood.

Host: **Ambystoma opacum, *Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea gutto-lineata, *Plethodon cinereus, *P. glutinosus, *P. metcalfi, *Pseudotriton r. ruber, *Triturus v. viridescens.*

First report from amphibians. So closely resembles *C. borreli* found in blood of freshwater fishes that it is considered the same species.

Family: Prowazekellidae Doflein, 1926

**Prowazekella longifilis* Alexieff, 1912. Rectum.

Syn.: Prowazekella sp. Mann, 1932; Das Gupta, 1935.

Host: **Ambystoma opacum*. Mann, 1932. Durham, North Carolina.
**Desmognathus f. fuscus*. *Ibid.*
**Eurycea bislineata cirrigera*. *Ibid.*
**Eurycea gutto-lineata*. *Ibid.*
**Plethodon glutinosus*. *Ibid.*
Ambystoma sp. Das Gupta, 1935. Maryland.
**Cryptobranchus alleganiensis, *Ambystoma maculatum, *Desmognathus o. ochrophaeus, *D. o. carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *Plethodon cinereus, *P. metcalfi, *P. yonahlossee, *Pseudotriton r. ruber, *P. m. montanus, *Triturus v. viridescens, *Gyrinophilus porphyriticus danielsi.*

Insufficient evidence for establishment of a new species. Described from newts and axolotls in 1911 by Alexieff, and later (1912) named by him. Form very variable.

Family: Trichomonadidae

**Tritrichomonas augusta* (Alexieff, 1911) Kofoid, 1920. Rectum.

Syn.: *Trichomonas augusta* Alexieff, 1911.
Trichomonas sp. Mann, 1932; Das Gupta, 1935.

Host: Ambystoma sp. Das Gupta, 1935. Maryland.
Ambystoma tigrinum. Ochoterena, 1921: 267. Mexico.
Triturus torosus. Kofoid and Swezy, 1915: 293; Tanabe, 1926: 129. California.

**Ambystoma opacum.* Mann, 1932. Durham, North Carolina.
**Desmognathus f. fuscus. Ibid.*
Eurycea bislineata cirrigera. Ibid.
**E. gutto-lineata. Ibid.*
**Triturus v. viridescens. Ibid.*
**Ambystoma maculatum, *Cryptobranchus alleganiensis, *Desmognathus o. ochrophaeus, *D. o. carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *Plethodon cinereus, *P. glutinosus, *P. metcalfi, *P. yonahlossee, *Pseudotriton r. ruber.*

Kofoid (1920) established the new name Tritrichomonas for all the trichomonad flagellates that have three anterior flagella.

Trichomonas prowazeki Alexieff, 1909. Rectum.

Syn: *Tetratrichomonas prowazeki* Kofoid and Swezy, 1915.

Host: *Triturus torosus.* Kofoid and Swezy, 1915: 341; Tanabe, 1926: 129. California.

Kofoid (1920) synonymizes Tetratrichomonas with Trichomonas.

Trichomitus parvus Swezy, 1915. Intestine.

Host: *Batrachoseps a. attenuatus.* Swezy, 1915: 89. California.
Triturus torosus. Ibid.
May be synonymous with Trichomonas (Wenyon, 1926: 676).

**Eutrichomastix batrachorum* Dobell, 1909. Rectum.

Syn.: Eutrichomastix sp. Mann, 1932.

Host: **Plethodon glutinosus.* Mann, 1932. Durham, North Carolina.
**Triturus v. viridescens. Ibid.*
**Ambystoma opacum, *Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *E. gutto-lineata, *Plethodon cinereus, *P. metcalfi, *P. yonahlossee, *Pseudotriton r. ruber.*
May be synonymous with Trichomonas (Chatton, 1920, Reichenow, 1918, 1920).

**Hexamastix batrachorum* Alexieff, 1912. Rectum.

Host: **Ambystoma opacum, *Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *E. gutto-lineata, *Gyrinophilus porphyriticus danielsi, *Plethodon cinereus, *P. glutinosus, *P. metcalfi, *Pseudotriton r. ruber, *Triturus v. viridescens.*

Order: Diplomonadida Wenyon, 1926

**Hexamitus batrachorum* Swezy, 1915. Rectum.

Host: *Aneides l. lugubris.* Swezy, 1915: 77. California.
Batrachoseps a. attenuatus. Ibid.
Ensatina eschscholtzii. Ibid.
**Desmognathus f. fuscus, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *E. gutto-lineata, *Plethodon cinereus, *P. glutinosus, *Triturus v. viridescens.*

**Hexamitus intestinalis* (Dujardin, 1841). Rectum.

Syn: *Hexamita intesinalis* Dujardin, 1841.
Octomitus intestinalis Prowazek, 1904.
Hexamita sp. Mann, 1932; Das Gupta, 1935.

Host: *Triturus torosus.* Swezy, 1915: 79. California.
Ambystoma sp. Das Gupta, 1935. Maryland.
**Triturus v. viridescens.* Mann, 1932. Durham, North Carolina.
**Ambystoma maculatum, *A. opacum, *Desmognathus f. fuscus, *D. o. carolinensis, *D. phoca, *D. quadramaculatus, *Plethodon cinereus, *P. glutinosus, *P. metcalfi, *Pseudotriton r. ruber.*
May invade blood of host (Danilewsky, 1889; Plimmer, 1914, 1916; Ponselle, 1919; Lavier and Galliard, 1925).

Hexamitus ovatus Swezy, 1915. Rectum.

Host: *Aneides l. lugubris.* Swezy, 1915: 73. California.
Batrachoseps a. attenuatus. Ibid.
Ensatina eschscholtzii. Ibid.
Triturus torosus. Ibid.: 74.

Class: Sporozoa Leuckart, 1879
Order: Coccidiomorpha Doflein, 1901
Sub-Order: Coccidia Leuckart, 1879
Family: Eimeriidae Poche, 1913

**Eimeria ranarum* (Labbe). Intestine and rectum.

Host: **Ambystoma opacum.*
Sub-Class: Cnidosporidia Doflein, 1901
Order: Myxosporidia Butschli, 1880
Sub-Order: Platysporea Kudo, 1919
Family: Myxobolidae Thelohan, 1892

**Myxobolus conspicuus* Kudo, 1919. Muscles.

Host: **Triturus v. viridescens.*
Insufficient evidence for establishment of new species.
Sub-Order: Haemosporidea Danilewsky, 1886
Family: Babesiidae Poche, 1913

**Cytamoeba bacterifera* Labbe, 1894. Erythrocytes.

Host: **Ambystoma maculatum, *A. opacum, *Desmognathus f. fuscus, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *E. gutto-lineata, *Plethodon cinereus, *P. glutinosus, *P. yonahlossee, *Pseudotriton r. ruber, *Triturus v. viridescens.*

Insufficient evidence for establishment of new species.

Family: Theileriidae

Dactylosoma jahni Nigrelli, 1929. Erythrocytes and erythroplastids.

Host: *Triturus v. viridescens.* Nigrelli, 1929, 1930. Pennsylvania.

Family: Haemogregarinidae Neveu-Lemaire, 1901

Haemogregarina riedyi (Labbe, 1898) Franca, 1917. Blood.

Syn.: *Haemopium riedyi* Labbe, 1889.

Host: *Batrachoseps a. attenuatus.* Labbe, 1898; Franca, 1917. California.

Sub-Phylum: Ciliophora Doflein, 1901

Class: Ciliata Perty, 1852

Sub-Class: Protociliata Metcalf, 1918

Order: Opalinata Stein, 1867

Family: Opalinidae Stein, 1860

Sub-Family: Protoopalininae Metcalf, 1920

Protoopalina mitotica (Metcalf, 1912). Rectum.

Syn.: *Opalina mitotica* Metcalf, 1912.

Host: *Ambystoma tigrinum.*. Metcalf, 1912: 79, 1923: 77. Nebraska.

Sub-Class: Euciliata Metcalf, 1918

Order: Holotrichida Stein, 1859

Sub-Order: Astomatea (Cepede, 1910)

Family: Discophryidae Cepede, 1910

**Haptophyrya gigantea* Maupas, 1879. Intestine

Host: **Plethodon glutinosus.*

**Haptophyrya michiganensis* Woodhead, 1928. Intestine.

Host: *Ambystoma jeffersonianum.* Woodhead, 1928: 177. Michigan.
Hemidactylium scutatum. Ibid.; Bush, 1933: 223, 1934: 251.
Eurycea bislineata cirrigera. Mann, 1932. Durham, North Carolina.
**E. gutto-lineata. Ibid.*
**Plethodon glutinosus. Ibid.*
**Ambystoma opacum, *Desmognathus f. fuscus, *D. phoca, *Eurycea bislineata wilderae, *Pseudotriton m. montanus.*

Order: Spirotrichida Butschli, 1889

Sub-Order: Heterotrichina Stein, 1859

Family: Plagiotomidae Poche, 1913

**Nyctotherus cordiformis* (Eherenberg, 1838). Rectum.

Host: **Triturus v. viridescens.*

Order: Peritrichida Stein, 1859

Family: Trichodinidae Claus

Trichodina pediculus Fulton, 1923. Gills and skin.

Host: *Necturus maculosus.* Fulton, 1923: 3; Charipper, 1929: 265. United States.

PLATYHELMINTHES

Class: Trematoda Rudolphi, 1808

Sub-Class: Monogenea Carus, 1863.

Order: Polyopisthocotylea Odhner, 1912

Family: Polystomidae van Beneden, 1858

Sphyranura oligorchis Alvey, 1933. Gills.

Host: *Necturus maculosus.* Alvey, 1933: 140; Alvey and Martin, 1934. Pennsylvania.

Sphyranura osleri Wright and MaCallum, 1887. Gills.

Host: *Necturus maculosus.* Wright and MaCallum, 1887: 1; Pratt, 1900: 659; Pearse, 1921: 6.

Sphyranura polyorchis Alvey, 1936. Gills.

Host: *Necturus maculosus.* Alvey, 1936. Pennsylvania.

Sub-Class: Digenea Carus, 1863

Order: Prosostomata Odhner, 1905

Sub-Order: Amphistomata Nitzsch

Family: Paramphistomidae Fischoeder, 1901

Sub-Family: Diplodiscinae Cohn, 1904

Megalodiscus Chandler, 1923.

Much uncertainty and disagreement has existed in regards to the validity of Megalodiscus. Chandler (1923) proposed this name for a new species that differed essentially from the type of Diplodiscus *(D. subclavatus)* as follows: in *D. subclavatus* the testes are single, the vitellaria extend in two groups from the pharyngeal region to the caudal end of the intestinal crura, and the posterior sucker has a cavity in its center instead of a prominence with a special musculature. As Harwood (1932) points out, the North American species never show any indication of fusion of the testes, the vitellaria are arranged in two or four groups, with the anterior follicles scarcely reaching the level of the anterior testis, and the posterior sucker has a prominence with special musculature. Dr. E. W. Price (personal communication) accepts Harwood's conclusions. It seems to the writer that the evidence requires the acceptance of Megalodiscus with the resulting synonomy. Harwood likewise pointed out that Holl's (1928) new species, *Opisthodiscus americanus,* agrees

in all respects with the characteristics of *Diplodiscus temperatus*, and should, therefore, with the above nomenclature, be considered as *Megalodiscus temperatus*. The writer is of the opinion that *M. americanus* and *M. temperatus* are synonymous, but until further study has been completed, shall maintain them here as valid species.

Megalodiscus americanus Chandler, 1923. Intestine.

Host: *Amphiuma means*. Chandler, 1923: 6. Louisiana.

**Megalodiscus intermedius* (Hunter, 1930) Harwood, 1932. Intestine.

Syn.: *Diplodiscus intermedius* Hunter, 1930.

Host: **Desmognathus f. fuscus*.

**Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932. Intestine.

Syn.: *Diplodiscus temperatus* Stafford, 1905.
Opisthodiscus americanus Holl, 1928.

Host: **Triturus v. viridescens*. Holl, 1928b: 176, 1932: 89; Mann, 1932; Kelley, 1934a. North Carolina and Pennsylvania.
**Ambystoma opacum*. Mann, 1932
**Desmognathus f. fuscus*.

Sub-Order: Distomata Leuckart, 1856
Family: Allocreadiidae Odhner, 1910
Sub-Family: Allocreadiinae Odhner, 1905

**Allocreadium pseudotritoni* Rankin, 1937. Intestine.

Host: **Pseudotriton m. montanus, P. r. ruber*. Rankin, 1937. North Carolina.

Sub-Family: Stephanophialinae Nicoll, 1909

Crepidostomum cooperi Hopkins, 1931. Intestine.

Host: *Necturus maculosus*. Van Cleave and Mueller, 1934: 203. New York.

Crepidostomum farionis (O. F. Mueller, 1784) Hopkins, 1934. Intestine.

Syn.: *C. laureatum* (Zeder) Braun, 1900.

Host: *Necturus maculosus*. Stafford, 1905; Heitz, 1917: 37. Canada.

Family: Gorgoderidae Looss, 1901
Sub-Family: Gorgoderinae Looss, 1889

Gorgodera amplicava Looss, 1899. Urinary bladder.

Syn.: *Distomum cynoides* Leidy, 1851.

Host: *Ambystoma maculatum*. Leidy, 1851, 1856. United States.
Pseudotriton m. montanus, P. r. ruber. Ibid.

Leidy reported this parasite as *D. cynoides* Zeder, 1800. Stafford (1903a) and Cort (1912) discuss and correct the nomenclature.

**Gorgoderina bilobata* Rankin, 1937. Urinary bladder.

Host: **Ambystoma opacum*, **Desmognathus f. fuscus*, ***Pseudotriton** *m. montanus*, *P. r. ruber*. Rankin, 1937. North Carolina.

Gorgoderina intermedia Holl, 1928. Urinary bladder.

Host: *Triturus v. viridescens*. Holl, 1928b: 180; Kelley, 1934a. North Carolina and Pennsylvania.

**Gorgoderina tenua* Rankin, 1937. Urinary bladder.

Host: **Eurycea gutto-lineata*. Rankin, 1937. North Carolina.

Phyllodistomum americanum Osborn, 1903. Urinary bladder.

Host: *Ambystoma tigrinum*. Osborn, 1903: 252; Lewis, 1935: 104. United States.

Phyllodistomum singulare Lynch, 1936. Urinary bladder.

Host: *Dicamptodon ensatus*. Lynch, 1936: 42. Oregon.

**Phyllodistomum solidum* Rankin, 1937. Urinary bladder.

Host: **Desmognathus f. fuscus*. Rankin, 1937. North Carolina

Family: Telorchiidae Stunkard, 1924

Sub-Family: Telorchiinae Looss, 1899

Perkins (1929) has shown that the European Telorchis is distinct from the American Cercorchis. Harwood (1932) found it necesary to transfer all of the North American species of Telorchis to Cercorchis and has been substantiated by later writers.

Cercorchis necturi Perkins, 1928. Intestine.

Host: *Necturus maculosus*. Perkins, 1928: 33. United States.

Cercorchis stunkardi (Chandler, 1923). Intestine.

Syn.: *Telorchis stunkardi* Chandler, 1923.

Host: *Amphiuma means*. Chandler, 1923: 4. Louisiana.

Cercorchis cryptobranchi McMullen and Roudabush, 1936. Intestine.

Host: *Cryptobranchus alleganiensis*. McMullen and Roudabush, 1936. Ames, Iowa.

Family: Plagiorchiidae Luhe, 1901

Sub-Family: Reniferinae Pratt, 1902

Manodistomum Stafford, 1905

Stafford established this genus for a single species, *M. occultum*. Price (1930) suggested that Plagitura Holl, 1928, was a synonym of Manodistomum. Harwood (1932) was unable to find any valid generic characteristics for the retention of Zeugorchis Stafford, 1905. Price (1936) redescribed Stafford's original specimens of *Z. aequatus*, and pointed out that many of the species described for this genus are not cogeneric with *Z.*

aequatus. Stunkard (1933) described *Plagitura parva* as a new species from Triturus. Kelley (1934) discussed the great variation occurring in this genus. Ingles (1933) and Stunkard (1936) described the life cycles of *Zeugorchis syntomentera* and *Plagitura parva*, respectively. As Stunkard points out "the experimental demonstration of the life cycles of *Z. syntomentera* and *P. parva* invalidates Harwood's opinion that both Plagitura and Zeugorchis are synonymous of Manodistomum," and that "since it is impossible at the present time to recognize specimens of *M. occultum* or to determine whether either Zeugorchis or Plagitura is synonymous with Manodistomum, all three generic names should be retained." Price (1935) concludes that *Z. syntomentera* is not cogeneric with *Z. aequatus*, and proposes the new genus Pseudorenifer for such forms, *Z. syntomentera*, therefore, becoming *P. syntomentera*.

Manodistomum occultum Stafford, 1905. Intestine.

Host: *Triturus v. viridescens*. Stafford, 1905. North America.

**Plagitura parva* Stunkard, 1933. Intestine.

Host: **Triturus v. viridescens*. Stunkard, 1933, 1936. Eastern United States.

**Plagitura salamandra* Holl, 1928. Intestine.

Host: **Triturus v. viridescens*. Holl, 1928, 1932; Harwood, 1932; Stunkard, 1933, 1936; Mann, 1932; Kelley, 1934, 1934a. Eastern United States and Texas.

**Ambystoma opacum*, **Eurycea gutto-lineata*.

Pseudorenifer syntomentera (Sumwalt, 1926) Price, 1935. Intestine.

Syn.: *Zeugorchis syntomentera* Sumwalt, 1926.

Host: *Triturus torosus*. Ingles, 1933: 169, 173. California.

Family: Dicrocoeliidae Looss, 1907

Sub-Family: Dicrocoeliinae

Mesocoelium americanum Harwood, 1932. Intestine.

Host: *Triturus meridionalis*. Harwood, 1932: 8. Texas.

Family: Brachycoeliidae Johnston, 1912

Sub-Family: Brachycoeliinae Looss, 1899

**Brachycoelium hospitale* Stafford, 1900. Intestine.

Syn.: *Distomum hospitale* Stafford, 1900.

D. (Brachycoelium) hospitale Stafford, 1903.

B. obesum Nicoll, 1914

B. daviesi Harwood, 1932.

B. meridionalis Harwood, 1932.

B. storeriae Harwood, 1932.

B. trituri Holl, 1928.

Host: **Plethodon cinereus.* Stafford, 1903: 824. Canada.
**Triturus v. viridescens.* Stafford, 1900: 403, 1902: 482, 1903: 824, 1905: 682; Cort, 1915: 198, 1919: 296; Holl, 1928b, 1932; Mann, 1932; Kelley, 1934a. United States.
Ambystoma texanum, Triturus meridionalis. Harwood, 1932. Texas.
**Ambystoma opacum, *Desmognathus f. fuscus, *Eurycea bislineata cirrigera, *Plethodon glutinosus.* Mann, 1932. North Carolina.
**Ambystoma maculatum, *Desmognathus phoca, *D. quadramaculatus, *D. o. carolinensis, *Eurycea b. wilderae, *E. gutto-lineata, *Plethodon metcalfi, *P. yonahlossee, *Pseudotriton r. ruber.*

A discussion of this genus with the above synonymy will be published in a separate paper.

Family: Cephalogonimidae Nicoll, 1909

Cephalogonimus amphiumae Chandler, 1923. Intestine.
Host: *Amphiuma means.* Chandler, 1923: 2. Louisiana.
Family: Heterophyidae Odhner
Sub-Family: Neochasminae Van Cleave and Mueller, 1932

Neochasmus umbellus Van Cleave and Mueller, 1932. Intestine.
Host: *Necturus maculosus.* Van Cleave and Mueller, 1934: 223. New York.
Family: Microphallidae (Ward, 1901) Travassos, 1920
Sub-Family: Microphallinae Ward, 1901

Monocaecum baryurum Stafford, 1903. Intestine.
Host: *Necturus maculosus.* Stafford, 1903. Canada.
Family: Strigeidae Railliet
Sub-Family: Diplostominae Monticelli, 1888

**Diplostomulum ambystomae* Rankin and Hughes, 1937. Body cavity.
Host: **Ambystoma opacum.* Mann, 1932. North Carolina.
**A. maculatum.*

**Diplostomulum desmognathi* Rankin, 1937. Body cavity.
Host: **Desmognathus f. fuscus, *D. phoca, *D. quadramaculatus.*

Diplostomulum trituri Shaw, 1934. Brain cavities and eyes.
Host: *Triturus v. viridescens.* Shaw, 1933: 132; Kelley, 1934a: 3. Pennsylvania.

*Metacercariae
Host: **Desmognathus f. fuscus, *D. quadramaculatus, *Pseudotriton r. ruber.*

Species inquirienda.

Distoma sp. Stiles and Hassall, 1894. Intestine.

Host: *Triturus v. viridescens.* Stiles and Hassall, 1894: 251. United States.

Further literature on this parasite has not been found. It is impossible to decide to what genus it belongs until the specimen has been restudied.

Class: Cestoidea Rudolphi, 1808
Order: Pseudophyllidea van Beneden, 1850
Family: Bothriocephalidae Blanchard, 1849

Bothriocephalus rarus Thomas, 1937. Intestine.

Host: *Triturus v. viridescens.* Thomas, 1927: 128; Kelley, 1934a. United States.

Family: Diphyllobothriidae Luhe, 1910
Sub-Family: Ligulinae Luhe, 1899

Ligulina intestinalis (Linnaeus, 1758). Intestine.

Host: Ambystoma sp. Cooper, 1919. Nebraska.
A. tigrinum. Ibid.

Order: Tetraphyllidea Schmarda, 1871
Family: Proteocephalidae LaRue, 1911

Woodland (1925) criticizes the characters on which LaRue (1914) bases his classification and points out that Ophiotaenia is synonymous with Crepidobothrium. Luhe (1899) showed that the generic name Proteocephalus, which has been applied to forms of this group, is invalid. Meggett (1927) distributes the species in the genus Ophiotaenia among the genera Crepidobothrium and Ichthyotaenia.

Crepidobothrium amphiumae Zeliff, 1932. Intestine.

Host: *Amphiuma tridactylum.*. Zeliff, 1932. Louisiana.

**Crepidobothrium cryptobranchi* (LaRue, 1914), Meggett, 1927. Intestine.

Syn.: *Ophiotaenia cryptobranchi* LaRue, 1914.

Host: *Cryptobranchus alleganiensis.* LaRue, 1914a. United States.
**Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Plethodon metcalfi, *Triturus v. viridescens.*

Crepidobothrium lonnbergii (Fuhrmann, 1895) Meggett, 1927. Intestine.

Syn.: *Ichthyotaenia lonnbergii* Fuhrmann, 1895.
Proteocephalus lonnbergii LaRue, 1909.
Ophiotaenia lonnbergii LaRue, 1911, 1914.

Host: *Necturus maculosus.* LaRue, 1909, 1911: 481, 1914: 16; Beddard, 1913; Johnston, 1914: 244; Pearse, 1921: 6; Canavan, 1928: 56; Stunkard, 1932: 163. Middle United States.

Ichthyotaenia filaroides (LaRue, 1909) Meggett, 1927. Intestine.

Syn.: *Proteocephalus filaroides* LaRue, 1909.
Ophiotaenia filaroides LaRue, 1911, 1914.

Host: *Ambystoma tigrinum.* LaRue, 1909: 17, 1911: 481, 1914: 209. Nebraska.
Ambystoma sp. Johnston, 1914: 244. North America.

*Crepidobothrium plerocercoids. Intestine.

Host: **Desmognathus phoca, *D. quadramaculatus, *Plethodon cinereus, *P. metcalfi, *Pseudotriton r. ruber.*

Ophiotaenia plerocercoids. Intestine.

Host: *Triturus v. viridescens.* Kelley, 1934a. Pennsylvania.

*Proteocephalid cysts. Wall of alimentary tract.

Host: *Ambystoma opacum, *Desmognathus f. fuscus, *Eurycea b. cirrigera, Plethodon glutinosus, *Triturus v. viridescens.* Mann, 1932. North Carolina.
**Desmognathus quadramaculatus, *Eurycea b. wilderae, *E. gutto-lineata, *Pseudotriton r. ruber.*
Order: Cyclophyllidea Braun, 1900
Family: Nematotaeniidae Luhe, 1910

Cylindrotaenia americana Jewell, 1916. Intestine.

Host: *Desmognathus f. fuscus.* Mann, 1932. North Carolina.

NEMATHELMINTHES

Class: Nematoda Rudolphi, 1808, emend. Diesing, 1861
Superfamily: Rhabditoidea Railliet, 1916
Family: Angiostomatidae R. Blanchard, 1895, emend. Lane, 1923

Angiostoma plethodontis Chitwood, 1933. Intestine.

Host: *Plethodon cinereus.* Chitwood, 1933: 511. Virginia.
Family: Rhabditidae Railliet, 1915

Rhabdias sp. larvae. Intestine and mesenteries.

Host: *Desmognathus f. fuscus, Eurycea b. cirrigera, E. gutto-lineata, Plethodon glutinosus, Triturus v. viridescens.* Mann, 1932. North Carolina.
Superfamily: Trichuroidea Railliet, 1916
Family: Trichuridae Railliet, 1915
Sub-Family: Capillariinae Railliet, 1915

Capillaria sp. intestine.

Host: *Triturus v. viridescens.* Kelley, 1934a. Pennsylvania.

Capillaria brevicollis Walton, 1935. Intestinal mucosa.

Host: *Triturus v. viridescens.* Walton, 1935: 43. Massachusetts.

**Capillaria inequalis* Walton, 1935. Intestinal mucosa.

Syn.: Capillaria sp. Mann, 1932. Holl, 1932.

Host: **Ambystoma opacum.* Mann, 1932; Walton, 1935: 44. North Carolina.

**Triturus v. viridescens.* Holl, 1932; Mann, 1932; Walton, 1935: 44. North Carolina.

**Desmognathus f. fuscus, *D. o. ochrophaeus, *D. o. carolinensis, *D. quadramacultaus, *Plethodon glutinosus.*

Capillaria tenua Mueller, 1932. Intestine.

Host: *Triturus v. viridescens.* Mueller, 1932. New York.

Superfamily: Strongyloides Weinland, 1858
Family: Trichostrongylidae Leiper, 1912
Sub-Family: Trichostrongylinae Leiper, 1908

Oswaldocruzia sp. Intestine.

Host: *Eurycea gutto-lineata.* Mann, 1932. North Carolina.

**Oswaldocruzia pipiens* Walton, 1929. Intestine.

Host: **Plethodon glutinosus.*

Oswaldocruzia subauricularis (Rudolphi, 1819) Travassos, 1917. Intestine.

Host: *Necturus maculosus.* Walton, 1935: 38. United States.

Superfamily: Dioctophymoidea Railliet, 1916
Family: Dioctophymidae Railliet, 1915

Eustrongylides wenrichi Canavan, 1929. Intestine.

Host: *Amphiuma means.* Canavan, 1929: 96, 1931: 207. Pennsylvania.

Superfamily: Oxyuroidea Railliet, 1916
Family: Oxyuridae Cobbold, 1864
Sub-Family: Oxyurinae Hall, 1916

Oxyuris (s. l.) *dubia* Leidy, 1856. Intestine.

Host: *Pseudotriton r. ruber.* Leidy, 1856; Walton, 1933: 8. United States.

**Oxyuris* (s. l.) *magnavulvaris* Rankin, 1937. Rectum.

Host: **Desmognathus f. fuscus, *D. ochrophaeus carolinensis, *D. phoca, *D. quadramaculatus, *Eurycea bislineata wilderae, *E. gutto-lineata, *Plethodon cinereus, *P. glutinosus, *P. yonahlossee, *Triturus v. viridescens.* Rankin, 1937. North Carolina.

Sub-Family: Cosmocercinae Railliet, 1916

**Cosmocercoides dukae* (Holl, 1928) Wilkie, 1930. Rectum.

Syn.: *Cosmocerca dukae* Holl, 1928.
Oxysomatium variabilis Harwood, 1930.
Cosmocerca variabilis (Harwood, 1930) Travassos, 1931.
Cosmocercoides variabilis Walton, 1933.

Host: *Ambystoma talpoideum.* Harwood, 1932: 51. Texas.
A. texanum. Ibid.
**A. opacum.* Mann, 1932; Walton, 1933: 15. North Carolina.
Triturus meridionalis. Harwood, 1930: 64; Walton, 1933: 15. Texas.
Triturus torosus. Ingles, 1936: 88. California.
**T. v. viridescens.* Holl, 1928a, 1932: 89; Harwood, 1932: 51; Walton, 1933: 11. North Carolina.
**Desmognathus f. fuscus,* **Plethodon cinereus,* **P. glutinosus.*

Aplectana sp. larvae. Intestine.

Host: *Ambystoma opacum, Plethodon glutinosus, Triturus v. viridescens.* Mann, 1932. North Carolina.

Sub-Family: Oxysomatiinae Railliet, 1916

Oxysomatium brevicaudatum (Zeder, 1800) Railliet and Henry, 1916. Intestine.

Syn.: *Oxysoma brevicaudatum* Zeder, 1800.

Host: *Pseudotriton r. ruber.* Stiles and Hassall, 1894: 342; Walton, 1933: 18. United States.

Family: Kathalaniidae (Travassos, 1918)
Sub-Family: Kathalaniinae Lane, 1914

Spironoura cryptobranchi Walton, 1930. Intestine.

Host: *Cryptobranchus alleganiensis.* Walton, 1930: 20, 1933: 5. United States.

Spironoura elongata. (Baird, 1858) Walton, 1932. Intestine.

Host: *Ambystoma tigrinum.* Walton, 1933: 5. Mexico.

Spironoura gracilis Leidy, 1856. Intestine.

Syn.: *Spirura gracilis* Perrier, 1897.

Host: *Ambystoma tigrinum.* Perrier, 1897: 1410; Walton, 1933: 5. United States.

Zanclophorus cryptobranchi Walton, 1930. Intestine.

Host: *Cryptobranchus alleganiensis.* Walton, 1930: 22, 1933. 4, 8; Baylis, 1933: 618. Pennsylvania.

Superfamily: Ascaroidea Railliet and Henry, 1915
Family: Heterocheilidae Railliet and Henry, 1915
Sub-Family: Anisakinae Railliet and Henry, 1912

Porrocaecum amphiumae (Leidy, 1856) Walton, 1927. Intestinal cysts.

Host: *Amphiuma means*. Walton, 1933: 3. United States.

Agamascaris odontocephala Steiner, 1924. Liver and stomach cysts.

Host: *Amphiuma tridactylum*. Walton, 1933: 3. United States.

Superfamily: Spiruroidea Railliet and Henry, 1915

Family: Spiruridae Oberley, 1885

Sub-Family: Spirurinae Railliet, 1916

Cystidicola sp. Walton, 1935. Cysts.

Host: *Eurycea bislineata bislineata*. Walton, 1935: 31. North Carolina.

Cystidicola stigmatura (Leidy, 1851). Stomach cysts.

Host: *Eurycea gutto-lineata*. Mann, 1932. North Carolina.

Spinitectus carolini Holl, 1928. Intestine.

Host: *Triturus v. viridescens*. Holl, 1928a: 184; Walton, 1935: 31. North Carolina.

*Spirurid cysts. Mesentery and intestinal wall.

Host: **Ambystoma opacum*, **Triturus v. viridescens*. Mann, 1932. **A. maculatum*, **Desmognathus f. fuscus*, **D. phoca*, **D. quadramaculatus*, **Eurycea gutto-lineata*, **Plethodon glutinosus*.

Sub-Family: Hedrurinae Railliet, 1916

Hedruris brevis Walton, 1930. Stomach wall.

Host: *Triturus v. viridescens*. Walton, 1930: 49, 1935: 32. United States.

Hedruris siredonis Baird, 1858. Stomach wall.

Host: *Ambystoma tigrinum*. Baird, 1858; Walton, 1935: 32. United States.

Triturus torosus. Chandler, 1919: 116; Walton, 1935: 32. North America.

Family: Acuariidae Seurat, 1913

Sub-Family: Acuariinae Railliet, Henry, and Sisoff, 1912

Acuaria sp. Stomach and intestinal cysts.

Syn.: Spiroptera sp. Walton, 1935.

Host: *Ambystoma opacum*, *Triturus v. viridescens*. Walton, 1935: 37. North Carolina.

Family: Gnathostomatidae Railliet, 1895, emend. Lane, 1923

Sub-Family: Spiroxyinae Baylis and Lane, 1920

Spiroxys allegheniensis Walton, 1930. Stomach.

Host: *Cryptobranchus alleganiensis*. Walton, 1930, 1935: 36. United States.

Spiroxys contortus Rudolphi, 1819. Stomach.

Host: *Triturus v. viridescens.* Hedrick, 1935. Michigan.

Family: Physalopteridae Leiper, 1908

*Physaloptera sp. larvae. Walton, 1935. Stomach.

Host: *Ambystoma opacum.* Mann, 1932. North Carolina.

**Desmognathus f. fuscus.* Mann, 1932; Walton, 1935: 33. North Carolina.

Plethodon glutinosus. Ibid.

**Pseudotriton m. montanus.*

Family: Camallanidae Railliet and Henry, 1915

*Camallanus sp. larvae. Intestine.

Host: **Triturus v. viridescens.*

Family: Cucullanidae Cobbold, 1864

Sub-Family: Seuratinae Hall, 1916

**Omeia papillocauda* Rankin, 1937. Rectum and intestine.

Host: **Desmognathus f. fuscus, *D. phoca, *D. quadramaculatus, *Gyrinophilus porphyriticus danielsi.* Rankin, 1937. North Carolina.

Superfamily: Filaroidea Weinland, 1858

Family: Filariidae (Cobbold, 1864) Claus, 1865

Sub-Family: Setariinae Yorke and Maplestone, 1926

Icosiella quadrituberculata (Leidy, 1856) Walton, 1927. Mesenteric cysts.

Host: *Amphiuma tridactylum.* Walton, 1935: 31. United States.

Sub-Family: Filariinae Stiles, 1907

*Filaria sp. larvae. Eyes.

Host: **Ambystoma opacum.*

Filaria s. l. sp. Walton, 1927. Cysts.

Host: *Ambystoma tigrinum.* Walton, 1935: 28. United States.

Filaria (s.l.) *cingula* v. Linstow, 1902. Skin.

Host: *Cryptobranchus alleganiensis.* Krecker, 1916: 74; Walton, 1935: 27. United States.

Family: Dracunculidae Leiper, 1912

Sub-Family: Dracunculinae (Stiles, 1907)

Philometra sp. cysts.

Host: *Triturus v. viridescens.* Holl, 1932: 89. North Carolina.

ACANTHOCEPHALA

Family: Echinorhynchidae Hamann, 1892

Acanthocephalus ranae (Schrank, 1788) Van Cleave, 1915. Intestine.

Syn: Echinorhynchus sp. Hassall, 1893; Stiles and Hassall, 1894.

Host: *Triturus v. viridescens.* Hassall, 1893; Stiles and Hassall, 1894: 358; Van Cleave, 1915: 175, 1919: 239. Maryland.

**Acanthocephalus acutulus* Van Cleave, 1931. Intestine.

Host: **Triturus v. viridescens.* Van Cleave, 1931: 46; Holl, 1932: 89. North Carolina.

**Ambystoma opacum,* **Plethodon glutinosus,* **Desmognathus f. fuscus.*

*Acanthocephalan cysts.

Host: **Desmognathus f. fuscus, Plethodon glutinosus.* Mann, 1932. **D. quadramaculatus.*

**Pomphorhynchus bulbicolli* Linkins, 1919. Intestine.

Host: **Triturus v. viridescens.* Leverett, Massachusetts.

ARTHROPODA

Class: Arachnida
Order: Acarina
Sub-Order: Trombidoidata
Family: Trombidiidae
Sub-Family: Trombiculinae

**Hannemania dunni* Sambon, 1928. Larvae encysted in skin.

Host: **Desmognathus f. fuscus.* Sambon, 1928; Mann, 1932. North America.

Eurycea b. cirrigera, **Plethodon glutinosus.* Mann, 1932.

**Ambystoma maculatum,* **A. opacum,* **E. gutto-lineata,* **Pseudotriton m. montanus.*

MOLLUSCA

Class: Lamellibranchia
Order: Eulamellibranchia
Family: Unionidae

Hemilastena ambigua Say. Gills.

Host: *Necturus maculosus.* Howard, 1915; Pearse, 1921. Mississippi, Wisconsin.

HOSTS

The following list includes all salamander hosts from North America for which parasites have been reported. The material on which this list

is based is derived from the preceding catalogue of parasites. The classification of hosts is based on that of Stejneger and Barbour (1933). An asterisk in front of a host and parasite name indicates that they have been encountered in the present study. For sake of convenience only the binomial name of each parasite is given. Reference to the parasite list will also give the author.

Class: Amphibia Linnaeus, 1858
Order: Caudata Oppel, 1811
Sub-Order: Proteida Cope, 1866
Family: Necturidae

Necturus maculosus maculosus (Raf.). Mudpuppy, Waterdog.
Syn.: *N. maculatus* Cope, 1889.
Protozoa: *Trichodina pediculus.*
Trematoda: *Cercorchis necturi, Crepidostomum cooperi, C. farionis, Monocaecum baryurum, Neochasmus umbellus, Sphyranura oligorchis, S. osleri, S. polyorchis.*
Cestoda: *Crepidobothrium lonnebergii.*
Nematoda: *Oswaldocruzia subauricularis.*
Mollusca: *Hemilastena ambigua.*
Sub-Order: Mutabilia Merrem, 1820
Family: Amphiumidae Garden, 1821

Amphiuma means Garden, 1821. Blind Eel, Congo Eel.
Trematoda: *Cephalogonimus amphiumae, Cercorchis stunkardi, Megalodiscus americanus.*
Nematoda: *Eustrongylides wenrichi, Porrocaecum amphiumae.*

Amphiuma tridactylum Cuvier, 1827.
Cestoda: *Crepidobothrium amphiumae.*
Nematoda: *Agamascaris odontocephala, Icosiella quadrituberculata.*
Family: Cryptobranchidae

**Cryptobranchus alleganiensis* (Daudin). Hell-bender.
Protozoa: **Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: *Cercorchis cryptobranchi.*
Cestoda: *Crepidobothrium cryptobranchi.*
Nematoda: *Filaria cingula, Spironoura cryptobranchi, Spiroxys allegheniensis, Zanclophorus cryptobranchi.*
Family: Salamandridae

Triturus meridionalis (Cope).
Trematoda: *Brachycoelium hospitale, Manodistomum occultum, Mesocoelium americanum.*
Nematoda: *Cosmocercoides dukae.*

Triturus torosus (Rathke). Giant newt, California newt.

Syn.: *Desmognathus torosus* Tanabe, 1926.
Diemyctylus torosus Cope, 1889.
Notophthalmus torosus Baird, 1849.

Protozoa: *Hexamitus intestinalis, H. ovatus, Trichomitus parvus, Karotomorpha swezi, Trichomonas prowazeki, Tritrichomonas augusta.*

Trematoda: *Pseudorenifer syntomentera.*

Nematoda: *Hedruris siredonis, Cosmocercoides dukae.*

**Triturus viridescens viridescens* (Raf.). Common newt.

Syn.: *Diemyctylus viridescens* Cope, 1889.

Protozoa: **Cryptobia borreli,* **Cytamoeba bacterifera, Dactylosoma jahni,* **Entamoeba ranarum,* **Euglenamorpha ranarum,* **Eutrichomastix batrachorum,* **Hexamastix batrachorum,* **Hexamitus batrachorum,* **H. intestinalis, H. ovatus,* **Karotomorpha swezi,* **Myxobolus conspicuus,* **Nyctotherus cordiformis,* **Prowazekella longifilis, Trichomitus parvus, Trichomonas prowazeki,* **Trichrichomonas augusta, Trypanosoma diemyctyli.*

Trematoda: **Brachycoelium hospitale, Diplostomulum trituri,* Distoma *sp.,* **Gorgoderina bilobata, G. intermedia, Manodistomum occulum,* **Megalodiscus temperatus,* *Metacercariae, **Plagitura parva,* **P. salamandra.*

Cestoda: *Bothriocephalus rarus,* **Crepidobothrium cryptobranchi,* Ophiotaenia plerocercoids, *Proteocephalid cysts.

Nematoda: Acuaria sp., Aplectana sp., *Camallanus sp., Capillaria sp., *C. brevicollis,* **C. inequalis, C. tenua,* **Cosmocercoides dukae, Hedruris brevis,* **Oxyuris magnavulvaris,* Philometra sp., Rhabdias sp., *Spinitectus carolini, Spiroxys contortus,* *Spirurid cysts.

Acanthocephala: **Acanthocephalus aculutus, A. ranae,* **Pomphorhynchus bulbicolli.*

Family: Ambystomidae

Ambystoma jeffersonianum (Green).

Protozoa: *Haptophrya michiganensis.*

**Ambystoma maculatum* (Shaw). Spotted salamander.

Syn.: *Amblystoma punctatum* Cope, 1889.
Salamandra maculata Leidy, 1851.

Protozoa: **Cytamoeba bacterifera,* **Euglenamorpha hegneri,* **Hexamitus intestinalis,* **Prowazekella longifilis,* **Tritrichomonas augusta.*

Trematoda: **Brachycoelium hospitale, *Diplostomulum ambystomae, Gorgodera amplicava, Phyllodistomum americanum.*
Acarina: **Hannemania dunni.*
Nematoda: *Spirurid cysts.

**Ambystoma opacum* (Gravenhorst). Marbled salamander.
Protozoa: **Cytamoeba bacterifera, *Cryptobia borreli, *Eimeria ranarum, *Eutrichomastix batrachorum, *Haptophrya michiganensis, *Hexamastix batrachorum, *Hexamitus intestinalis, *Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale, *Diplostomulum ambystomae, *Gorgoderina bilobata, *Megalodiscus temperatus, *Plagitura salamandra.*
Cestoda: *Proteocephalid cysts.
Nematoda: *Acuaria* sp., *Aplectana* sp., **Capillaria inequalis, *Cosmocercoides dukae, *Filaria* sp., *Physaloptera* sp., *Spirurid cysts.
Acanthocephala: **Acanthocephalus acutulus.*
Acarina: *Hannemania dunni.*

Ambystoma talpoideum (Holbrook). Mole salamander.
Nematoda: *Cosmocercoides dukae.*

Ambystoma texanum (Matthes).
Syn.: *A. microstomum* Stejneger and Barbour, 1923.
Trematoda: *Brachycoelium hospitale.*
Nematoda: *Cosmocercoides dukae.*

Ambystoma tigrinum (Green). Tiger salamander, Axolotl.
Protozoa: *Karotomorpha swezi, K. bufonis, Protoopalina mitotica, Tritrichomonas augusta.*
Trematoda: *Phyllodistomum americanum.*
Cestoda: *Ichthyotaenia filaroides, Ligula intestinalis.*
Nematoda: Filaria sp., *Hedruris siredonis, Spironoura elongata, S. gracilis.*

Ambystoma sp.
Protozoa: *Hexamitus intestinalis, Karotomorpha ambystomae, Prowazekella longifilis, Tritrichomonas augusta.*
Cestoda: *Ichthyotaenia filaroides, Ligula intestinalis.*

Dicamptodon ensatus (Eschscholtz).
Trematoda: *Phyllodistomum singulare.*
Family: Plethodontidae Gray, 1850

Batrachoseps attenuatus attenuatus (Eschscholtz).
Syn.: *B. alternatus* Swezy, 1916.
Protozoa: *Haemogregarina riedyi, Hexamitus batrachorum, H. ovatus, Karotomorpha swezi, Trichomitus parvus.*

Hemidactylium scutatum (Schlegel). Four-toed salamander.
Protozoa: *Haptophrya michiganensis.*

**Plethodon cinereus* (Green). Red-backed salamander.
Syn.: *P. erythronotus* Stafford, 1903.
Protozoa: **Cryptobia borreli*, **Cytamoeba bacterifera*, **Eutrichomastix batrachorum*, **Hexamastix batrachorum*, **Hexamitus batrachorum*, **H. intestinalis*, **Prowazekella longifilis*, **Karotomorpha swezi*, **Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Cestoda: *Crepidobothrium plerocercoids.
Nematoda: *Angiostoma plethodontis*, **Cosmocercoides dukae*, **Oxyuris magnavulvaris.*

**Plethodon glutinosus* (Green). Slimy salamander.
Protozoa: **Cryptobia borreli*, **Cytamoeba bacterifera*, **Eutrichomastix batrachorum*, **Haptophrya gigantea*, **H. michiganensis*, **Hexamastix batrachorum*, **Hexamitus batrachorum*, **H. intestinalis*, **Karotomorpha swezi*, **Prowazekella longifilis*, **Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Cestoda: *Proteocephalid cysts.
Nematoda: Aplectana sp., **Capillaria inequalis*, **Cosmocercoides dukae*, **Oswaldocruzia pipiens*, **Oxyuris magnavulvaris*, Physaloptera, sp., Rhabdias sp., *Spirurid cysts.
Acanthocephala: **Acanthocephalus acutulus.* Cysts.
Acarina: **Hannemania dunni.*

**Plethodon metcalfi* Brimley.
Protozoa: **Cryptobia borreli*, **Eutrichomastix batrachorum*, **Hexamastix batrachorum*, **Hexamitus intestinalis*, **Karotomorpha swezi*, **Prowazekella longifilis*, **Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Cestoda: **Crepidobothrium cryptobranchi*, Crepidobothrium plerocercoids.

**Plethodon yonahlossee* Dunn.
Protozoa: **Cytamoeba bacterifera*, **Eutrichomastix batrachorum*, **Karotomorpha swezi*, **Prowazekella longifilis*, **Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Nematoda: **Oxyuris magnavulvaris.*

Ensatina eschscholtzii Gray.
Syn.: *Plethodon oregonensis* Cope, 1889.
Protozoa: *Hexamitus batrachorum*, *H. ovatus*, *Monocercomonas melolonthae.*

**Gyrinophilus porphyriticus danielsi* (Blatchley).

Protozoa: **Hexamastix batrachorum, *Prowazekella longifilis.*
Nematoda: **Omeia papillocauda.*

**Pseudotriton montanus montanus* (Baird).

Protozoa: **Haptophrya michiganensis, *Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: **Allocreadium pseudotritoni, *Gorgoderina bilobata, Gorgodera amplicava.*
Nematoda: *Physaloptera sp.
Acarina: **Hannemania dunni.*

**Pseudotriton ruber ruber* (Sonnini).

Syn.: *Salamandra ruber* Sonnini, 1802.
Spelerpes ruber Cope, 1889.
Protozoa: **Cryptobia borreli, *Cytamoeba bacterifera, *Eutrichomastix batrachorum, *Hexamastix batrachorum, *Hexamitus intestinalis, *Karotomorpha swezi, *Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: **Allocreadium pseudotritoni, *Brachycoelium hospitale, *Gorgoderina bilobata, Gorgodera amplicava,* *Metacercariae.
Cestoda: *Crepidobothrium plercercoids, *Proteocephalid cysts.
Nematoda: *Oxysomatium brevicaudatum, Oxyuris dubia.*

Eurycea bislineata bislineata (Green).

Nematoda: Cystidicola sp.

**Eurycea bislineata cirrigera* (Green).

Protozoa: *Haptophrya michiganensis, *Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Cestoda: *Proteocephalid cysts.
Nematoda: Rhabdias sp.
Acarina: *Hannemania dunni.*

**Eurycea bislineata wilderae* Dunn.

Protozoa: **Cytamoeba bacterifera, *Eutrichomastix batrachorum, *Haptophrya michiganensis, *Hexamastix batrachorum, *Hexamitus batrachorum, *Prowazekella longifilis, *Tritrichomonas augusta.*
Trematoda: **Brachycoelium hospitale.*
Cestoda: *Proteocephalid cysts.
Nematoda: **Oxyuris magnavulvaris.*

**Eurycea gutto-lineata* (Holbrook).

Protozoa: **Cryptobia borreli, *Cytamoeba bacterifera, *Eutrichomastix batrachorum, *Haptophrya michiganensis, *Hexamastix batrachorum, *Hexamitus batrachorum, *Prowazekella longifilis, *Tritrichomonas augusta.*

Trematoda: **Brachycoelium hospitale, *Gorgoderina tenua, *Plagitura salamandra.*

Cestoda: *Proteocephalid cysts.

Nematoda: *Cystidicola stigmatura,* Oswaldocruzia sp., **Oxyuris magnavulvaris, Rhabdias* sp., *Spirurid cysts.

Acarina: *Hannemania dunni.*

Eurycea longicauda (Green).

Protozoa: *Karotomorpha swezi.*

Aneides lugubris lugubris (Hallowell).

Protozoa: *Hexamitus batrachorum, H. ovatus.*

**Desmognathus fuscus fuscus* (Raf.). Dusky salamander.

Protozoa: **Cryptobia borreli, *Cytamoeba bacterifera, *Eutrichomastix batrachorum, *Haptophrya michiganensis, *Hexamastix batrachorum, *Hexamitus batrachorum, *H. intestinalis, *Karotomorpha swezi, *Prowazekella longifilis, *Tritrichomonas augusta.*

Trematoda: **Brachycoelium hospitale, *Diplostomulum desmognathi, *Gorgoderina bilobata, *Megalodiscus intermedius, *M. temperatus,* *Metacercariae, **Phyllodistomum solidum.*

Cestoda: **Crepidobothrium cryptobranchi, Cylindrotaenia americana,* *Proteocephalid cysts.

Nematoda: **Capillaria inequalis, *Cosmocercoides dukae, *Omeia papillocauda, *Oxyuris magnavulvaris,* *Physaloptera sp., Rhabdias sp., *Spirurid cysts.

Acanthocephala: **Acanthocephalus acutulus,* *Cysts.

Acarina: **Hannemania dunni.*

**Desmognathus ochrophaeus ochrophaeus* (Cope).

Protozoa: **Prowazekella longifilis, *Tritrichomonas augusta.*

Nematoda: **Capillaria inequalis.*

**Desmognathus ochrophaeus carolinensis* Dunn.

Protozoa: **Cryptobia borreli, *Eutrichomastix batrachorum, *Hexamastix batrachorum, *Hexamitus intestinalis, *Karotomorpha swezi, *Prowazekella longifilis, *Tritrichomonas augusta.*

Trematoda: **Brachycoelium hospitale.*

Cestoda: **Crepidobothrium cryptobranchi.*

Nematoda: **Capillaria inequalis, *Oxyuris magnavulvaris.*

**Desmognathus phoca* (Matthes).

Protozoa: **Cryptobia borreli*, **Cytamoeba bacterifera*, **Eutrichomastix batrachorum*, **Haptophrya michiganensis*, **Hexamastix batrachorum*, **Hexamitus batrachorum*, **H. intestinalis*, **Karotomorpha swezi*, **Prowazekella longifilis*, **Tritrichomonas augusta*.

Trematoda: **Brachycoelium hospitale*, **Diplostomulum desmognathi*.

Cestoda: **Crepidobothrium cryptobranchi*, *Crepidobothrium plercercoids.

Nematoda: **Omeia papillocauda*, **Oxyuris magnavulvaris*, *Spirurid cysts.

**Desmognathus quadramaculatus* (Holbrook).

Protozoa: **Cryptobia borreli*, **Cytamoeba bacterifera*, **Eutrichomastix batrachorum*, **Hexamastix batrachorum*, **Hexamitus batrachorum*, **H. intestinalis*, **Karotomorpha swezi*, **Prowazekella longifilis*, **Tritrichomonas augusta*.

Trematoda: **Brachycoelium hospitale*, **Diplostomulum desmognathi*, *Metacercariae.

Cestoda: **Crepidobothrium cryptobranchi*, *Crepidobothrium plerocercoids, *Proteocephalid cysts.

Nematoda: **Capillaria inequalis*, **Omeia papillocauda*, **Oxyuris magnavulvaris*, *Spirurid cysts.

Acanthocephala: *Cysts.

APPENDIX 2: KEYS TO PARASITES OF NORTH AMERICAN SALAMANDERS

PROTOZOA

Practically all salamanders examined had protozoan parasites. These occur most commonly in the rectum, some species, however, inhabit the whole lower digestive tract. A few have been found in the blood, both in plasma and red corpuscles. Encystment in body muscles occurs in very few cases. All of the following protozoa have been recorded from North American salamanders. An asterisk following the name indicates that the species has been observed in the present study.

Key to Protozoan Parasites

1 (10) In blood 2
2 (5) In plasma 3
3 (4) Size small, 20-25 microns long, 2 flagella....*Cryptobia borelli**
4 (3) Size large, 40-50 microns long, 1 flagellum
Trypanosoma diemyctyli
5 (2) In erythrocytes 6

6 (7) Elongate, slender, doubling on themselves, occupying large part of cell; 2 in each cell ..... *Haemogregarina riedyi*
7 (6) Small, more or less spherical, located at one end of cell 8
8 (9) Spherical, 3-7 microns in diameter; stain red *Cytamoeba bacterifera**
9 (8) Elongate, 9 x 2 microns; stain blue ..... *Dactylosoma jahni*
10 (1) Not in blood 11
11 (12) Encysted in muscles, cysts ½-4 mm. in diameter, containing ellipsoidal spores, 9-11.5 x 6.5-8 microns *Myxobolus conspicuus**
12 (11) Not encysted in muscles, parasites of intestine 13
13 (16) Possessing pseudopodia or oocysts 14
14 (15) Pseudopodia ..... *Entamoeba ranarum**
15 (14) Ovoid oocysts, 17 x 12 microns, with 4 sporocysts each with 2 sporozoites ..... *Eimeria ranarum**
16 (13) Possessing cilia or flagella 17
17 (26) Ciliated forms 18
18 (25) Entire body ciliated 19
19 (20) Body kidney-shaped, a notch on middle of right side; esophagus; 60-120 microns long ..... *Nyctotherus cordiformis**
20 (19) Body cylindrical or elongate, no notch or esophagus 21
21 (24) Body elongate, circular sucker on ventral anterior extremity provided with two rows of cilia 22
22 (23) Body long, 1.1-1.6 mm., usually with many long segments *Haptophyra michiganensis**
23 (22) Body short and squat, segments short ... *Haptophyra gigantea**
24 (21) Body cylindrical, 0.3 x 0.037 mm., circular in cross-section, 10 large chromosomes ..... *Protoopalina mitotica*
25 (18) Body with 2 rows of cilia, one around anterior, other around posterior end; body flatly cone-shaped ... *Trichodina pediculus*
26 (17) Flagellate forms 27
27 (28) With green chromatophores and bright red stigmata *Euglenamorpha hegneri**
28 (27) No green chromatophores 29
29 (30) 2 flagella, one projecting forwards 4x body length, other backwards 2x body length; tapering posterior, blunt anterior end *Prowazekella lonifilis**
30 (29) More than 2 flagella 31
31 (46) Axostyle present 32
32 (35) Undulating membrane present 33
33 (34) 3 anterior flagella ..... *Tritrichomonas augusta**
34 (33) 4 anterior flagella ..... *Trichomonas prowazeki*
35 (32) Undulating membrane absent 36

36 (41) 2 axostyles; 6 anterior, 2 posterior flagella 37
37 (38) Proximal ends of axostyles unite and pass to blepharoplast complex as a broad band and then divide again, ½ going to each half of the blepharoplast ..... *Hexamitus intestinalis**
38 (37) Proximal ends of axostyles not united 39
39 (40) 2 groups of chromatin granules at posterior end of axostyles *Hexamitus ovatus*
40 (39) Axostyles without posterior granules ..*Hexamitus batrachorum**
41 (36) 1 axostyle; anterior flagella only 42
42 (45) 4 anterior flagella 43
43 (44) Axostyle along surface of body ..*Monocercomonas melolonthae*
44 (43) Axostyle in center of protoplasm *Eutrichomastix batrachorum**
45 (42) 6 anterior flagella .....*Hexamastix batrachorum**
46 (31) Axostyle absent 47
47 (48) 3 anterior flagella .....*Trichomitus parvus*
48 (47) 4 anterior flagella 49
49 (52) Surface of body with oblique striations 50
50 (51) Flagella of equal length .....*Karotomorpha ambystomae*
51 (50) 1 long and 1 short flagellum from each basal granule *Karotomorpha swezi**
52 (49) Surface of body without striations .....*Karotomorpha bufonis*

TREMATODA

The adult digenetic trematodes of salamanders occur most commonly in the digestive tract. Three genera, Phyllodistomum, Gorgodera, and Gorgoderina, inhabit the urinary bladder, while a larval fluke, Diplostomulum, lives in body and brain cavities. Only one genus of trematodes belonging to the subclass Monogenea has been found, Sphyranura, on gills and skin.

Key to Trematode Parasites

1 (6) Living on skin or gills; suckers at posterior end of body provided with hooks (Order: Monogenea) 2
2 (3) 20-23 testes .....*Sphyranura polyorchis*
3 (2) Less than 18 testes 4
4 (5) 12-16 testes .....*Sphyranura osleri*
5 (4) 5-7 testes .....*Sphyranura oligorchis*
6 (1) Living in internal organs; no posterior suckers with hooks (Order: Digenea) 7
7 (12) Immature forms, genetalia represented by a small mass of cells; muscular holdfast organ behind acetabulum; fore and hind body distinct; in body or brain cavities or eye 8
8 (9) In brain cavities or eyes .....*Diplostomulum trituri*
9 (8) In body cavity 10

10 (11) Elongate; only 2 transverse commissural vessels in excretory granule system and these without dendritic branches; corpuscles with but a single concretion. In Ambystoma sp. *Diplostomulum ambystomae**

11 (10) Spherical; 4 transverse commissural vessels with dendritic branches; 2-4 concretions in each corpuscle. In Desmognathus sp. ..... *Diplostomulum desmognathi**

12 (7) Adult forms, well-developed genetalia; no holdfast organ; in alimentary canal of host 13

13 (14) Intestine of but a single caecum..... *Monocaecum baryurum*

14 (13) Intestine forked, resulting in 2 caeca 15

15 (16) With a prominent complete circle of spines surrounding the mouth ..... *Neochasmus umbellus*

16 (15) Mouth without complete circle of spines 17

17 (22) Powerful acetabulum at posterior end 18

18 (21) Horn-shaped, narrow at anterior, wide at posterior end; simple pharyngeal pockets 19

19 (20) Small testes; vitellaria extending from level of posterior testis to end of caeca ..... *Megalodiscus temperatus**

20 (19) Relatively large testes; vitellaria extending around posterior ends of caeca ..... *Megalodiscus americanus*

21 (18) Clove-shaped, no change in width from region of esophagus to posterior sucker; reticular pharyngeal pockets *Megalodiscus intermedius**

22 (17) Acetabulum not at posterior end 23

23 (28) Body leaf-like, divided into disc-like hindbody and a narrow cylindrical forebody. In urinary bladder 24

24 (27) Ovary and vitellaria lobed; testes large 25

25 (26) Stylet-glands retained in mature individual; crura short *Phyllodistomum singulare*

26 (25) Stylet-glands lost; crura long..... *Phyllodistomum americanum*

27 (24) Ovary and vitellaria smooth; testes small *Phyllodistomum solidum**

28 (23) Body elongate 29

29 (36) Large acetabulum divides narrow forebody from tubular, sac-like hindbody; in urinary bladder 30

30 (31) 9 testes; short ..... *Gorgodera amplicava*

31 (30) 2 testes; elongate 32

32 (33) Ventral sucker about twice size of oral *Gorgoderina intermedia*

33 (32) Suckers about equal 34

34 (35) Thick uterine folds between acetabulum and ovary; vitellaria two-lobed ..... *Gorgoderina bilobata**

35 (34) No thick uterine folds between acetabulum and ovary; vitellaria usually four-lobed ..... *Gorgoderina tenua**

36 (29) Body not divided by large acetabulum 37

37 (42) Skin smooth; vitellaria of follicles extending from level of pharynx to, and filling, posterior end of body; uterus between caeca and anterior to testes 38

38 (41) Oral sucker surrounded by papillae 39

39 (40) Genital pore anterior to crural fork; 4 dorsal smaller than 2 ventral papillae ..... *Crepidostomum farionis*

40 (39) Genital pore posterior to crural fork; papillae about equal in size ..... *Crepisdostomum cooperi*

41 (38) Oral sucker without papillae ..... *Allocreadium pseudotritoni**

42 (37) Skin spiny; vitellaria never as in 37; uterus in various positions 43

43 (48) Genetalia close to acetabulum, within caecal limits 44

44 (45) Genital pore in front of oral sucker *Cephalogonimus amphiumae*

45 (44) Genital pore in front of acetabulum 46

46 (47) Ovary anterior to testes; caeca never extend posterior to acetabulum ..... *Brachycoelium hospitale**

47 (46) Ovary posterior to testes; caeca extend to midbody length *Mesocoelium americanum*

48 (43) Genetalia near posterior end 49

49 (54) Testes located against ends of caeca 50

50 (51) Narrow behind; pharyngeal glands present; esophagus short *Pseudorenifer syntomentera*

51 (50) Narrow in front; no pharyngeal glands; esophagus long .....52[1]

52 (53) Size large; sexually mature at 1.5 mm. ..*Plagitura salamandra**

53 (52) Size small; sexually mature at 0.78 mm., gonads relatively larger and situated more posteriorly in body than in *P. salamandra* *Plagitura parva**

54 (49) Testes located at posterior end of body, tandem, within caecal limits 55

55 (56) Vitellaria extending to slightly beyond anterior level of acetabulum, 8-10 follicular groups on right side, 11-12 on left *Cercorchis cryptobranchi*

56 (55) Vitellaria not reaching level of acetabulum 57

57 (58) Acetabulum larger than oral sucker; vitellaria distinctly lobate, 10 on right side, 9 on left ..... *Cercorchis stunkardi*

58 (57) Acetabulum and oral sucker equal; vitellaria follicles in groups, 20-40 to a group ..... *Cercorchis necturi*

[1] *Manodistomum occultum* Stafford should be included here. As Stunkard (1936) points out, however, since it is "impossible to determine whether Statfford had one or more species, it is impossible to identify or recognize *M. occultum*."

CESTODA

The cestodes of salamanders are most commonly found in the digestive tract. In addition to their serving as definitive hosts, salamanders shelter larval tapeworms which probably reach sexual maturity in birds or mammals. Only two orders of cestodes have been recorded, the Bothriocephaloidea (commonly known as the Pseudophyllidea) and the Tetraphyllidea.

Key to Cestode Parasites

1 (4) Scolex with a single terminal, or with 2 opposite sucking organs, never with 4 suckers 2

2 (3) Eggs thin-shelled, without lid; uterine pore ventral; cirrus and vagina open dorsal and posterior to uterine pore; scolex distinctly elongate .....*Bothriocephalus rarus*

3 (2) Eggs thick-shelled, with lid; cirrus and vagina open on same surface as, and anterior to, uterine pore; scolex short, not set off from rest of worm ... .*Ligula intestinalis*

4 (1) Scolex with 4 sucking organs 5

5 (12) Vitellaria with numerous follicles distributed on each side in longitudinal marginal zone, rarely in the entire surface zone of the proglottid 6

6 (7) 4 suckers supplemented with a fifth, or apical organ, in the center .....*Crepidobothrium cryptobranchi**

7 (6) No apical organ present 8

8 (11) Vagina always anterior to cirrus pouch 9

9 (10) Ovary solid ..... *Crepidobothrium amphiumae*

10 (9) Ovary of anastomosing tubules .....*Ichthyotaenia filaroides*

11 (8) Vagina anterior or posterior to cirrus pouch
Crepidobothrium lonnbergii

12 (5) Vitellaria condensed, in a single mass, usually immediately posterior to ovary; one testis in each proglottid
Cylindrotaenia americana

NEMATODA

Parasitic nematodes are found in considerable numbers in salamanders. Some species are extremely abundant and occur as frequently as any other parasite. They occur generally in the digestive tract, with occasional species living in body tissues. Some nematodes in mesenteries and body organs are encysted, using salamanders as intermediate hosts.

Key to Nematode Parasites

1 (4) Esophagus with a prebulbar swelling 2

2 (3) 3 simple lips; esophagus terminates in a pseudobulb
Angiostoma plethodontis

3 (2) 6 insignificant lips; esophagus terminates in a bulb
Rhabdias sp.

4 (1) Esophagus without a prebulbar swelling 5
5 (10) Esophagus consisting of a narrow tube running through the center of a row of single cells for most of its length 6
6 (9) Para-esophageal cells less than 80 7
7 (8) Size large, female 13.7 mm. long; head-vulva distance 4.5 mm. *Capillaria inequalis**
8 (7) Size small, female 6.6 mm. long; head-vulva distance 2.6 mm. *Capillaria brevicollis*
9 (6) Para-esophageal cells 90-110; size intermediate; head-vulva distance 3.35 mm. ..... *Capillaria tenua*
10 (5) Esophagus not consisting of a narrow tube running through the center of a row of single cells 11
11 (16) Males with a bursa copulatrix 12
12 (15) Bursa copulatrix cuticular and supported by rays 13
13 (14) Dorsal bursal ray terminates in a pair of simple lateral branches and a medium branch with digitations *Oswaldocruzia subauricularis*
14 (13) Dorsal bursal ray terminates in 4 branches, inner pair of which is again bifurcated ..... *Oswaldocruzia pipiens**
15 (12) Bursa copulatrix muscular, not supported by rays *Eustrongylides wenrichi*
16 (11) Males without bursa copulatrix 17
17 (34) Esophagus dilated posteriorly into a bulb usually containing a denticular apparatus and frequently separated from the rest of the esophagus by a constriction 18
18 (19) Females with stout vulval papilla .... *Oxyuris magnavulvaris**
19 (18) Females without stout vulval papilla 20
20 (27) Males without any special development of ventral precloacal musculature 21
21 (22) Males with a single spicule ..... *Oxyuris dubia*
22 (21) Males with 2 spicules 23
23 (26) Gubernaculum present 24
24 (25) Males with plectanes ..... *Cosmocercoides dukae**
25 (24) Males without plectanes ..... Aplectana sp.
26 (23) Gubernaculum absent ..... *Oxysomatium brevicaudatum*
27 (20) Males with precloacal musculature strongly developed in the form of a sucker or pseudosucker 28
28 (29) Lips united to one another by a horseshoe-shaped cuticular band; vestibule large and wide; pharynx absent *Zanclophorus cryptobranchi*
29 (28) Lips not united by a horseshoe-shaped cuticular band; vestibule small; pharynx present 30

30 (31) Base of lip with a collar-like chitinized ring
Spironoura cryptobranchi
31 (30) Base of lip without collar-like chitinized ring 32
32 (33) Each lip with 2 blunt papillae and an inner tripartite, heavily cuticularized tooth .....*Spironoura elongata*
33 (32) Each lip with 2 inner and 2 outer papillae
Spironoura gracilis
34 (17) Esophagus not dilated posteriorly into a bulb 35
35 (38) Head with three large lobes or lips; body relatively short 36
36 (37) Immature; no intestinal caecum ...*Agamascaris odontocephala*
37 (36) Adult; intestinal caecum present ....*Porrocaecum amphiumae*
38 (35) Head without 3 large lobes or lips but with 2 lateral lips of 4 or 6 small lips, or lips absent; body filiform 39
39 (40) With 6 lateral lips, semi-fused to 3 .....*Omeia papillocauda**
40 (39) With less than 6 lateral lips 41
41 (58) Usually with 2 lateral lips; chitinous buccal cavity or vestibule usually present; vulva usually in the middle of the body or posterior to it 42
42 (45) Males always rolled about females, the posterior end of which is invaginated forming a sucker-like groove from which projects a chitinous hook 43
43 (44) With distinct longitudinal striations in both sexes; form short
Hedruris brevis
44 (43) No distinct longitudinal striations; form elongate
Hedruris siredonis
45 (42) Males not rolled about females; no posterior sucker-like groove with hook 46
46 (47) With large chitinous buccal capsule. .....Camallanus sp.*
47 (46) Without large chitinous buccal capsule 48
48 (49) Anterior end of body with cuticular cordons .....Acuaria sp.
49 (48) Anterior end of body without cuticular cordons 50
50 (53) Mouth with 2 large lateral tri-lobed lips each with the cuticle of its inner surface dentigerous; with a cuticular headbulb 51
51 (52) Size large; distinct longitudinal striations
Spiroxys alleghenicnsis
52 (51) Size small; no striations .....*Spiroxys contortus*
53 (50) Lips without dentigerous cuticle; no cuticular headbulb 54
54 (55) Large cephalic collarette present .....Physaloptera sp.*
55 (54) Cephalic collarette absent 56
56 (57) Cuticular spines present .....*Spinitectus carolini*
57 (56) Cuticular spines absent .....*Cystidicola stigmatura*
58 (41) Usually without lips; buccal capsule absent or rudimentary; vulva in esophageal region 59

59 (60) Females enormously larger than males; vulva atrophied in gravid female ..... Philometra sp.
60 (59) Females at most 3 or 4 times as long as males; vulva not atrophied in gravid female 61
61 (62) Mouth surrounded by 4 small spinous teeth, with 4 papillae external to these ..... *Icosiella quadritubercula*
62 (61) Mouth without 4 spinous teeth or external papillae 63
63 (64) Adult form; in skin ..... *Filaria cingula*
64 (63) Immature form ..... Filiaria sp.*

ACANTHOCEPHALA

Only three acanthocephalan parasites have been reported from North American salamanders. These live as adults in the lumen of the digestive tract. Immature forms have been found in the body cavity.

Key to Acanthocephalan Parasites

1 (2) Distinct bulb in neck, between body and proboscis *Pomphorhynchus bulbicolli**
2 (1) No bulb in neck 3
3 (4) Proboscis armed with 12-20 longitudinal rows of 4-7 hooks each; longest hooks 77-80 microns long *Acanthocephalus ranae*
4 (3) Proboscis with 18-24 longitudinal rows of 9-12 hooks each; longest hooks 40-47 microns long ... *Acanthocephalus acutulus**

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SOME ASPECTS OF THE ROLE OF WATER IN INSECT HIBERNATION

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INTRODUCTION AND LITERATURE

For many years the lethal action of unfavorable low temperatures has been studied intensively, but the rôle of water during hibernation has not been fully appreciated until rather recently. This condition has existed because the results of the action of temperature are frequently more easily measured and because of the many intrinsic factors which influence the insect water balance. The relation, however, between the water content of insects and moisture conditions of their hibernacula is of very great importance.

Several investigators have demonstrated that changes in the water balance before and during hibernation are factors which are associated with the ability of insects to endure the unfavorable low temperatures of winter. Bachmetjew (1901) observed that the supercooling and freezing of insects were influenced by the "sap coefficient," or the percentage of water in the total body weight of the insect. Payne (1927, 1927a, 1928) studied the effect of desiccation on the undercooling and freezing points of oak borers, and the survival of the Japanese beetle at low temperatures. This author found that a partial dehydration of the tissues produced an effect which caused the experimental animals to be more cold-hardy. In the case of the Japanese beetle, she says, "Dehydration of larvae is associated with cold hardiness to the quantity factor of low temperature as well as to the intensity factor of low temperature." Furthermore, it was observed that dehydration beyond two-thirds of the body weight decreases cold hardiness to the quantity factor of low temperature. Sacharov (1930) arrived at the conclusion that the degree of cold resistance is related to the total water content, or more especially to the balance between easily freezable water and fat in the insect body. Robinson (1927, 1928) advanced our knowledge of the relation of water to the survival of hibernating insects by his investigations of the importance of hydrophilic colloids in reducing the amount of freezable water. Uvarov (1931) has pointed out the possibility that fat may represent one of those hydrophilic colloids which regulate the balance between bound and free water (freezable water in Sacharov's experiments).

Several workers have found that commonly there is a loss of water associated with hibernation. Breitenbecker (1911, 1918) and Fink (1925) have observed a marked decrease in the amount of total water in the Colorado potato beetle when that insect approached the period of hibernation. Bodine (1921, 1923) has demonstrated a similar change in water content of hibernating grasshoppers. The percentage of water decreased during hibernation and increased when the animals became active again. Payne

(1927a) recorded the change in the water content of various insects during hibernation. In all cases during dormancy, there was a decrease in the percentage of water which was associated with an increase in the cold-hardiness of the various species. Holmquist (1928) has concluded from his study of the ant *Formica ulkei* that the loss of water from this species is more or less rhythmical because individuals kept at room temperature showed a reduction in water content even though they were surrounded with moist soil. The loss of water in these animals was not great, however, and the normal balance was regained as early as January. The author attributed such a behavior to cyclic changes in metabolism and suggested that it is not merely the effect of temperature and humidity.

The observations just mentioned have been the result of investigations concerning the cold-hardiness of insects normally exposed to rather severe winter conditions. One might expect them to undergo certain adjustments which would enable them to resist the action of cold weather. Certain authors, Robinson (1926, 1928), Payne (1927), and Sacharov (1930), have demonstrated that insects such as stored-products pests, aquatic insects, and the honey bee are unable to modify their water balance to satisfy the demands for protection against low temperatures. Robinson found that the granary weevil, *Sitophilus granarius*, showed a drop in the total water content during an exposure to a moderately low temperature, but also there was a drop in the percentage of bound water and an increase in the percentage of free water. This change was accompanied by an elevation of the freezing point of the free water and less resistance to low temperature. Thus it is evident that the loss of free water is not alone sufficient to increase the ability of a species to protect itself against winter mortality.

Within recent years there have been many careful investigations of the effect of atmospheric moisture upon the development and survival of insects. Particularly important studies have been made in the laboratories of Buxton, Janisch, and Zwölfer; however, there have been relatively few attempts to evaluate the effect of environmental moisture on insect survival during the winter. Escherich (1912) studied the effect of atmospheric moisture on the mortality of the young larvae of *Lymantria monacha* at low temperatures. His experiments were not very extensive but they show that the young larvae are more easily killed by low temperatures after being exposed in moist air. Breitenbecker (1911, 1918) observed that a cool, dry environment is necessary for the potato beetle to enter hibernation in the proper physiological condition. If individuals of the hibernating generation were kept moist and warm, they would enter a partial dormancy, and there would be a high mortality. Breitenbecker (1918) has stated, furthermore, that in arid regions the length of the period of hibernation is dependent upon the available moisture, whereas temperature seems to be more im-

portant in temperate areas. He has also concluded that the higher percentage of individuals surviving hibernation in clay soil than in sand is due to the greater chance for excessive evaporation from the sandy soil. Robinson (1926) exposed an unexpected relation between the water content of grain and one of its inhabitants, the granary weevil. These beetles had a lower percentage of water when they lived in moist grain than when they were reared in dry grain. Apparently, the beetles in dry grain attempted to compensate for the moisture deficiency by the release of some water which had been bound by the colloidal particles in the body and by the production of the water of metabolism. Payne (1929) has made an interesting contribution to our knowledge of the effects of atmospheric moisture on hibernation: In studying certain insects which can resist low temperature, she found that the survival of tussock moth eggs bears a linear relation to absolute humidity. Cousin (1932) has stated that the prepupae of *Lucilia sericata* will not enter a diapause if they are exposed to a saturated atmosphere and that such treatment also lowers the resistance of the prepupae to low temperatures. Zwölfer (1933) has studied the relation of temperature and humidity to the survival of the various stages of development of the nun moth. Because of technical difficulties, no experimental work was done at low temperatures, but he states the following with regard to the effect of relative humidity during hibernation, "Hinsichtlich der Luftfeuchtigkeit dürfte die Grenze des optimalen Bereiches für das überwinterende Ei zwischen jener während der herbstlichen Übergangsperiode und der Schlüpfzeit in Frühjahr hegen, also schätzungsweise bei 60-70%." Recently Decker and Andre (1936) have shown that dehydration produced by brief exposures at low relative humidities increased the cold resistance of chinch bugs.

That contact moisture may be very important has been suggested by Babcock (1924, 1927). He determined that the successful hibernation of the corn-borer, *Pyrausta nubilalis*, is very dependent upon environmental moisture. This author concluded, "Hibernating larvae are very sensitive to available moisture throughout the period, and it is essential that the moisture requirement be supplied by actual contact. High air humidity will not suffice." In this case there has been no suggestion of a relation between moisture and freezing. He continues, "As the period of dryness during hibernation is lengthened, the mortality during the spring correspondingly increases." Babcock believes that dormancy in this insect is essentially a "preparation" period for pupation, and it is the accomplishment of the latter which is prevented by insufficient moisture. Zwölfer (1927) agrees that moisture is necessary for the successful pupation of the corn-borer, but he has stated (1930) that the fully developed larvae seek dry hibernation quarters.

Fenton and Owen (1931) also found contact moisture favorable for the hibernation of the pink boll worm, *Pectinophora gossypiella*. The greatest

survival of the larvae was in a silty-clay soil, the least in a sandy soil. In either type of soil the highest percentage of larvae survived when the soil was irrigated. Other similar records might be reported, but they are chiefly concerned with desert or subtropical species for which desiccation is the most important factor during hibernation.

Among the widely scattered references to the mode of hibernation of various insects are found statements with regard to the effect of contact moisture on freezing. Stone (1930), in his discussion of the bionomics of some Tabanidae, says: "They cannot survive in frozen soil, at least if it is moist, as the writer discovered when trying to imitate natural conditions." The procedure of the experiment was not disclosed and so it is impossible to value this contribution. Miller (1931) observed that the larvae of the western pine beetle are much less resistant to cold when they have become dormant due to the saturation of bark with water. Decker and Andre (1936) have studied the effect of contact water on the low temperature resistance of the chinch bug. They have concluded that freezing in ice is absolutely fatal and that probably the compression due to the stress forces in the ice is responsible for death.

All of the experimental work does not indicate a harmful effect of contact moisture. Sacharov (1930), and Parker (1930), and Salt (1937) found that grasshopper eggs were as resistant to low temperature when moist as when dry. Likewise, Arnim (1936) observed no significant difference in the hardiness of nun moth eggs when they were exposed dry, soaked in water, or enclosed in ice. The eggs of the forest tent caterpillar are also unaffected by the presence of contact moisture when exposed to low temperatures.

Students of animal communities who have examined the seasonal activities of insects have made observations which suggest that environmental moisture may play an important part in the distribution of invertebrates hibernating in a temperate climate. Weese (1924), Blake (1926), Holmquist (1930), and Park (1930) all have remarked about the probability of the importance of water in the selection of hibernation quarters. In addition, Hancock (1923) has stated, with regard to some hibernating ichneumonidae, that the majority of species prefer a damp situation, whether it be under bark or in tufts of grass. Hamilton (1885) reported the discovery of the larvae of *Dectes spinosus* in stems of rag-weed where they were often surrounded by a cylinder of ice. *Lixus concavus* was found embedded in ice in a patch of *Rumex* on low ground. King (1895) remarked about the finding of *Camponotus pennsylvanicus* embedded in ice in the decayed portions of hollow trees. Holmquist (1928) opened badly decayed logs and found clusters of the fly *Pyrellia serena* surrounded by ice crystals.

On the contrary, several investigators have studied species of insects

which seem to show a preference for dry situations during hibernation. Spett (1925) followed the seasonal activity of species of the genus *Saprinus* and found that these beetles prefer dry sand in which to spend the winter. In the fall they crawl down through the dry sand to the edge of the wet sand where they are not exposed to severe low temperatures. Dennys (1927) has made the following statement with regard to insects hibernating in central British Columbia: "Some of the insects showed no great aversion to the damper situations, such as those usually found under heavy bark near the ground level; however, for the majority of hibernating insects it would seem imperative that they find absolutely dry spots in which to pass the winter." Plath (1927) observed the selection of hibernation quarters by bumblebee queens. They usually chose a north exposure and avoided damp places. Likewise, Zwölfer (1930) recorded the observation that the larvae of the corn-borer select fully matured, dry cornstalks for hibernation instead of the more moist, younger stalks.

It has already been mentioned that moisture is essential for the initiation of pupation in the corn-borer. The emergence of the potato beetle from hibernation is likewise dependent upon suitable moisture conditions, according to Breitenbecker (1918). He also states that the adult beetles can absorb moisture from the air when the relative humidity is high. Townsend (1926) has proposed a theory for the breaking up of hibernation in the codling moth which is based upon the observed effects of contact moisture on this species. When the larvae were given periodic soakings, the time which elapsed before pupation was greatly reduced. Douglas (1928, 1933) has found that contact moisture is essential for the spring emergence of the Mexican bean beetle, *Epilachna corrupta*. He found that, although permanent emergence rarely occurs when the temperature is below a certain minimum, rainfall is the initial stimulus and that temperature merely accelerates the emergence of the bettle.

During the past few years the author has had the opportunity to make insect population studies in several types of environments during the period of insect hibernation. In the course of this work the fact became obvious that many hibernating insects were existing under apparently unfavorable conditions. One must conclude from the majority of the reports reviewed above that insects are favored by a loss of water and a reduction in the amount of free water. However, it has been observed that a considerable number of species in hibernation were exposed to very moist media. The purpose of this investigation is to examine the nature of this seemingly paradoxical situation and to determine the action of environmental moisture on the dormant individuals of several species of insects.

SURVEY OF THE STAGES OF DEVELOPMENT IN WHICH SOME IMPORTANT ECONOMIC PESTS HIBERNATE AND THE TYPES OF HIBERNACULA

Blatchley (1895), in his notes on winter collecting in Indiana, shows a great preponderance of adult individuals representing the numerous species of hibernating insects. He collected, especially, three orders in which hibernation is very common in the adult stage, namely Orthoptera, Hemiptera, and Coleoptera. Holmquist (1926) collected hibernating Arthropods in forests near Chicago, Illinois. This author made a very intensive study and concluded that insects may hibernate in only a single stage. An examination of his data shows that the most commonly occurring stage is the adult; however, he admits the possibility of missing the eggs of many species. Bremer (1928) realized the importance of winter mortality in the course of insect outbreaks, and also the fact that its control value was not the same for different species. A study of his data on some of the most important European insect pests shows that those species which hibernate in an inactive stage, such as the egg or pupa, are the most resistant to winter influences.

It was believed that it might be of some interest and value to survey the published accounts regarding the hibernation habits of some important economic pests. For this purpose 159 species of insects were investigated in the literature. The results of this research are tabulated in Table 1. It can be seen that certain groups of species may pass the winter more commonly in stages other than that of the adult. Only the Coleoptera and Hemiptera show the highest percentage of species hibernating in the adult stage. It is significant that the greatest percentage of the species included in this survey enter hibernation in a motile state. The importance of this fact will be discussed in connection with the selection of hibernation quarters.

An examination of the records contained in the work of Holmquist (1928) shows that most of the several species which he found were located in such protected places, as in fallen logs, in the soil, and under leaves or trash. The notes of Blatchley show a similar condition. Weese (1924), Blake (1926), and Park (1930) observed that the stratification which occurred in forests during the seasons of activity had disappeared during the winter. The majority of animals were present in or near the level of the soil. The data listed in Table 1 agrees well with these observations. The majority of the economic pests considered choose similarly sheltered places in which to hibernate. Only inactive stages such as eggs and pupae are found exposed to winter cold in the majority of the species.

The actual amount of protection which is afforded by the various types of hibernacula is a subject which has received relatively little critical study. Holmquist (1931) has investigated the temperatures of the microclimates

associated with insect hibernacula. He made continuous records of the temperature in logs, under logs and in leaves, and compared them with the air temperature. His conclusions were that such places as rotten logs and dead leaves offer considerable protection against low temperature. In his experiments the temperatures of the hibernacula remained near or at 0°C. and did not drop below the normal undercooling points of hibernating insects. Payne (1927) placed an oak log in a chamber in which a low temperature of —40°C. could be maintained. Temperatures were taken under loose bark, moderately tight bark, and tight bark. The results of this experiment indicated that although the bark offers little absolute insulation, it does cause the under-bark temperatures to lag behind that of the environment.

TABLE 1. Developmental Stages and Types of Hibernacula.

Orders and Stages	No. Species	Percentage	Percentage in Various Hibernation Quarters*										
			1	2	3	4	5	6	7	8	9	10	11
Lepidoptera	51												
Eggs		11.7				16.6		83.4					
Larvae		45.1	8.7		4.3		13.1	17.4	4.4		8.7		47.8
Pupae		43.1				18.2					59.1		22.7
Adults		7.8											100.0
Coleoptera	40												
Eggs		2.5										100.0	
Larva		47.5	10.5				36.8			31.6	10.5	5.3	5.3
Adults		65.0								11.5	7.6	7.6	80.7
Homoptera	24												
Eggs		75.0		33.3	11.1			55.5					
Nymphs		16.6					25.0			25.0		50.0	
Adults		25.0		33.3				16.6					66.6
Diptera	16												
Larvae		50.0		25.0						37.5	25.0		12.5
Pupae		37.5									100.0		
Adults		12.4							100.0				
Hemiptera	8												
Eggs		25.0	100.0										
Adults		75.0											100.0

*(1) In living plants, (2) on living plants, (3) in dead plants, (4) on dead plants, (5) in living trees, (6) on living trees, (7) in dead trees or fallen logs, (8) in soil, below level of severe frost, (9) upper soil, (10) base of plants (stalks or roots), (11) soil surface under leaves or trash.

A brief series of hibernacula temperature readings were made in several environments near St. Paul. Table 2 shows clearly that there may be a very important temperature gradient between the air and the place where insects were found. The temperatures were taken with a standardized mercury thermometer which was placed in holes bored three inches into logs and which was merely thrust into the leaves and trash on the surface of the ground. At the time when these observations were made there was no snow on the ground or the gradient would have been much greater.

TABLE 2. Temperature Gradient Between the Air and Some Hibernacula.

Date	Hibernaculum	Air Temperature	Temperature of Hibernaculum	Difference
12/26/33	Oak log (early stage of decay)	—14.5°C.	— 8.3°C.	6.2°C.
12/28/33	Oak log (early stage of decay)	—27.2	—12.5	14.7
1/ 1/34	Oak log (early stage of decay)	—18.3	—10.0	8.3
12/26/33	Oak log (late stage of decay)	—16.8	—12.3	4.5
12/28/33	Oak log (late stage of decay)	—29.2	—18.6	10.6
1/ 1/34	Oak log (late stage of decay)	—20.0	—14.8	5.2
12/26/33	Fallen leaves on ground	—15.5	— 8.6	6.9
12/28/33	Fallen leaves on ground	—28.1	—11.6	6.5
1/ 1/34	Fallen leaves on ground	—15.9	— 9.8	6.1

Many workers have reported the effective insulation which is provided by snow; they have been able to show that insect abundance can be correlated with the depth of snow during severe winters. The work of Criddle (1917), Newcomer (1920), Barber (1924), Sacharov (1930), and Mail (1930, 1932) may be cited in this regard. Soil temperature records taken at the University of Minnesota during the winter of 1933-1934 illustrate the importance of even a light covering of snow. Table 3 contains representative data which has been selected to show the difference between the air temperature and that of the soil at a depth of two inches.

TABLE 3. Effect of Snow Cover on Soil Temperature.

Date	Inches Snow	Air Temperature	Soil Temperature	Difference
2/21/34	0	— 7.0°C.	— 5.5°C.	2.5
2/22/34	0	—12.0	— 8.5	3.5
2/23/34	0	—13.0	—10.0	3.0
2/24/34	—	—16.0	—12.5	3.5
11/15/33	1	—10.0	0.0	10.0
11/16/33	1	—11.5	— 1.0	10 5
1/ 1/34	Trace	—11.5	— 5.5	6.0
1/ 2/34	3.5	— 7.0	— 4.5	2.5
1/ 3/34	3.0	— 9.5	— 3.0	6.5

It is unfortunate that the depth of snow was not recorded during the previous year because the effects of snow as an insulator were much more marked. The small difference between the air temperature and that of the soil on the second day of January is due to the fact that it was colder on the previous day and that there always is a considerable lag in the soil temperature. During the winter of 1934-1935 similar records show that the soil temperature at a two-inch depth could be as high as —4°C. when the air temperature was —28°C. In this case the ground was covered with 10 inches of snow.

MOISTURE CONDITIONS IN THE FIELD WITH SPECIAL REFERENCE TO THE HIBERNACULA OF VARIOUS INSECTS

In the course of the seasonal changes during the periods of prehibernation and hibernation, meteorological factors do exert a considerable influence on moisture conditions in the field. The data compiled in Table 4 have been taken from Bulletin W. of the U. S. Weather Bureau, and the figures represent the mean value of observations which have been carried on for many years in St. Paul, Minnesota.

TABLE 4. Climatic Factors which Influence Moisture Conditions in the Field.

Month	Mean Temperature °C.	Mean Relative Humidity	Mean Absolute Humidity	Saturation Deficiency	Mean Per cent Sunlight
January	—10.95	79	1.5956	0.4242	49
February	— 8.89	79.5	1.8787	0.4845	56
March	— 1.67	73.5	2.9893	1.0779	55
April	+ 7.78	64.5	5.0848	2.7986	58
May	+14.22	63.5	7.6440	4.3940	58
June	+19.44	67.5	11.2930	5.437	61
July	+22.22	67.0	13.3129	6.557	70
August	+21.00	69.5	12.8339	5.632	65
September	+16.11	71.5	9.6596	3.851	59
October	+ 9.22	71.0	6.1514	2.513	52
November	+ 0.11	74.5	3.4032	1.165	45
December	— 7.22	79.0	2.1225	0.5515	41

It is quite evident from the above figures that the factors which hasten the dissipation of moisture are less important during the fall and winter than during the spring and summer. A glance at the table will show that there is a gradual decrease in temperature, saturation deficiency, and the percentage of sunlight; at the same time there is a slight increase in the percentage of relative humidity. Such a condition tends to slow up the rate of the evaporation of water from the soil and other places in which insects hibernate. It will be noticed that the absolute humidity decreases as winter approaches. However, it is believed that this factor may be of little importance to the insect because with the coincident decrease in temperature, there is a corresponding increase in the ability of hygroscopic materials to absorb water. The values for the absolute humidity and the saturation deficiency are expressed as vapor pressure in millimeters of mercury.

During the fall and winter of 1933 samples were taken from various places in which insects were hibernating. These samples were collected in tin pill boxes and brought to the laboratory to be weighed. The material was dried in an oven, which was held at a constant temperature of 105°C. until there was no appreciable loss in weight. After the desiccated sample had cooled, it was weighed again and the moisture content was calculated. In the case of several representative samples the hygroscopic coefficient was determined. This value was obtained by placing an oven-dried sample in

a glass desiccator in which a saturated atmosphere could be maintained. The sample was weighed frequently and allowed to remain in the desiccator at 22°C. until there was no further gain in weight. Both the water content and hygroscopic coefficient of the samples listed in Table 5 are expressed in terms of the percentage of the dry weight of the materials. The collections were made in several localities during the fall, and the geographical locations in the table may be identified by the following key: (1) Coon Creek, Ramsey County, Minnesota; (2) St. Croix Falls, Wisconsin; (3) Fort Snelling Military Reservation; (4) Rice Lake, Anoka County, Minnesota; (5) Rose Hill, Anoka County, Minnesota. In addition, the following symbols have been used to abbreviate the names of the micro-habitats in which the insects were found: Lv—under or in leaves; So—in soil; B—under bark; Lo—in log; U. Lo.—under log; B. T.—base of tree; C—cavities in logs.

Two important facts have been exposed by this examination of typical hibernation quarters, even though there is a lack of negative evidence. In the first place, it is obvious that the hibernacula of a great variety of invertebrates is very moist during the time when insects and other anthropods are reacting to the autumnal thermal decline. The percentage of water in all but a few of the samples exceeded the hygroscopic coefficient to such an extent that water could actually be expressed from much of the material. It is easy to conclude from these facts that the insects were exposed to an atmosphere saturated with water and were often in actual contact with liquid water. Lebedeff (1928) has proved that the relative humidity of soil air is always at the saturation point if the soil contains moisture in an amount greater than the maximum hygroscopicity. There can be little doubt that the same condition exists in other hygroscopic materials, such as leaves and wood, because moisture becomes available for evaporation after the point of maximum hygroscopicity has been passed. In many cases it was not necessary to assume that the inhabitants of a hibernaculum had been exposed to a high humidity because individuals were often found with ice crystals on their bodies or immediately surrounding them. Clusters of the fly, *Pyrellia serena,* were removed from cells in rotten logs when there were drops of water on their bodies and when the cells were completely lined with hoar frost. Holmquist (1928) has reported this same discovery and states that the cavities are always moist and often frozen. Many of the insect larvae were imbedded in ice under the bark of logs. In most cases there was a perceptible space between the body of the insect and the ice. A close examination showed that this space was filled with a liquid. The nature of the liquid was not determined but it is assumed that it was water. Robinson (1928) has demonstrated that the dormant insects can maintain a body temperature higher than that of the environment, and it is possible that freezing of this liquid was prevented by the body heat of the larvae.

TABLE 5. The Water Content of Various Hibernacula and Some of the Most Common Residents.

Date	Collection No.	Locality	Community	Hibernaculum	Per cent water	Hygroscopic coefficient	Most common species
9/19/33	1	(1)	Oak Savanna	B.	89.7	26.5	*Camponotus herculeanus pennsylvanicus*, larvae of Elateridae and Pyrochroidae
9/19/33	2	(1)	Oak Savanna	So.	6.7	1.8	Larvae of Scarabaeidae
9/19/33	3	(1)	Flood-plain	Lo.	392.1	32.4	Pyrochroidae (larvae), *Zonitoides* (snail), Mycetophilidae, immature earthworms and slugs
9/25/33	4	(1)	Flood-plain	B.	276.8	....	Larvae of Mycetophilidae and Tipulidae
9/25/33	5	(1)	Flood-plain	Lo.	115.3	26.2	*Camponotus herculeanus pennsylvanicus*, larvae of Elateridae, Centipedes and earthworms
9/25/33	6	(1)	Flood-plain	Lo.	406.2	36.8	Earthworms
9/25/33	7	(1)	Flood-plain	B.	146.9	....	Pyrochroidae (larvae)
10/22/33	8	(1)	Flood-plain	So. U. Lo.	40.2	5.46	*Goniodiscus* sp., Centipedes and several species of the genus *Harpalus*
10/22/33	9	(1)	Flood-plain	C.	210.0	14.9	*Vespula maculata*, *Vespula diabolica*
11/20/33	10	(1)	Flood-plain	So. U. Lo.	18.7	5.8	Occasional leafhopper
11/20/33	11	(1)	Flood-plain	C. B.	133.2	....	*Amblyteles* sp.
11/20/33	12	(1)	Flood-plain	Lo.	120.7	25.9	*Camponotus herculeanus pennsylvanicus*
11/20/33	13	(1)	Flood-plain	So. Lv.	48.7	7.3	Aggregated Bibionid larvae
11/20/33	14	(1)	Flood-plain	Lo.	62.1	29.3	None
10/19/33	15	(4)	Tamarack Bog	So.	774.0	28.9	Few dipterous larvae
10/19/33	16	(4)	Tamarack Bog	So.	316.6	25.3	*Lasius niger*, lepidopterous pupae, and pupa of *Lygaeonematus erichsonii*
11/24/33	17	(2)	Maple–Basswood	B.	286.1	24.6	Larvae of Elateridae and Pyrochroidae
11/24/33	18	(2)	Maple–Basswood	B.	147.2	....	Larvae of Thereviidae
11/24/33	19	(2)	Maple–Basswood	C.	400.0	30.1	Clusters of *Pyrellia serena*
11/24/33	20	(2)	Oak	Lo.	221.3	26.1	Larvae of Tenebrionidae
11/24/33	21	(2)	Maple–Basswood	So.	68.6	17.3	Larvae of Tipulidae and Syrphidae
10/15/33	22	(2)	Maple–Basswood	Lv.	67.1	21.6	Tipulidae and Spiders
10/15/33	23	(2)	Maple–Basswood	C. B.	338.0	24.3	*Amblyteles* sp.
10/15/33	24	(2)	Maple–Basswood	Lv.	72.1	13.8	Leafhoppers and Collembola
11/17/33	25a	(3)	Oak savanna	B. T. Lv.	74.2	11.6	*Ceratomegilla fuscilabris*, *Leptocoris trivittatus*
11/17/33	25b	(3)	Oak savanna	So.	18.1	8.3	Same as above
11/17/33	26	(3)	Oak savanna	So. B. T.	20.4	....	*Ceratomegilla fuscilabrie*, *Hypodamia convergens*, *Alsophila pometaria*
11/17/33	27	(3)	Oak savanna	So. B. T.	11.9	4.6	Numerous *Leptocoris trivittatus*
12/ 1/33	28a	(4)	Oak savanna	So.	42.3	5.6	*Lygus pretensis* and leafhoppers
12/ 1/33	28b	(4)	Oak savanna	Lv.	46.4	....	*Lygus pretensis*, leafhoppers, *Nabis* sp.

During January, 1934, a thaw occurred which produced much surface water. A sharp drop in temperature followed this thaw, and many insects, which were hibernating in leaves and on the ground, were imprisoned in ice. A collection of *Lygus pretensis,* which were found in such a condition, was warmed and the insects soon became active. Thus it is evident that some species can endure moisture conditions which appear to be unfavorable for the necessary loss of freezable water.

The second fact of paramount importance for this discussion is that the inhabitants of these wet hibernation quarters were not merely those which neither could nor would escape from their situation but were also species which, apparently, had made a definite choice. Insects which are included in this latter category are the wasps of the genus *Amblyteles,* the beetles, *Ceratomegilla fuscilabris* and *Hippodamia convergens,* and others. Two of these species were observed as they sought shelter when the temperature was falling. The coccinellid beetle, *Ceratomegilla fuscilabris,* seems to be very deliberate as it searches through leaves and trash on the ground. Individuals crawl about upon grass stems and leaves and then disappear from sight for a moment as they investigate the soil below. This process is continued until a favorable spot has been found. The action of the fly *Pyrellia serena* is very similar as it investigates first one old insect burrow and then others until a selection has been made. The question of preference has been investigated and will be reported in the next section.

THE MOISTURE PREFERENCES OF INSECTS ENTERING HIBERNATION

Observations in the field suggested the idea that certain species of insects might possibly exert some choice or make a tropic response with regard to the moisture in available hibernacula. In order to test the theory that insects are not obliged to spend the winter with moist surroundings but that they show some positive preference the following species were studied: The beetles, *Ceratomegilla fuscilabris* and *Hippodamia convergens;* the bugs, *Leptocoris trivittatus* and *Lygus pretensis;* the fly, *Pyrellia serena;* and the ant *Camponotus herculeanus pennsylvanicus.* With the exception of the latter, all of the species belong to the group which move from one environmental stratum to another upon entering hibernation. The insects were tested in a wire screen cage, one foot square. The cage had a board floor which was divided into four equal parts with narrow, movable strips of wood. Samples of soil having moisture contents of zero per cent, 10 per cent, 20 per cent and 30 per cent were introduced into the compartments at the beginning of the experiment. The soil in each compartment was covered with a thin layer of leaves, and in each case the soil and leaves had the same moisture content. The arrangement of the different soils was varied, in most cases so that the position in the cage would have no in-

fluence. After the introduction of the artificial forest floor a counted number of test animals was scattered at random on the leaves. When they had distributed themselves throughout the cage, it was placed in a low temperature cabinet, and the temperature was allowed to drop very slowly from room temperature or above, to a point at or near zero Centigrade. The results of the several experiments are included in Table 6.

TABLE 6. Moisture Preference of Various Insects.

	Experiment Number	Number Individuals	Percentage Found in Each Section			
			0 per cent	10 per cent	20 per cent	30 per cent
Ceratomecilla fuscilabris	1	100	2.1	3.2	71.2	23.4
	2	100	5.3	18.0	56.3	20.2
	3	100	12.2	19.4	40.8	27.5
	4	75	5.6	7.0	66.2	21.1
Hippodamia convergens	5	75	11.1	34.6	38.2	16.0
	6	100	7.3	32.8	41.6	19.3
Leptocoris trivittatus	7	50	34.1	29.5	22.7	13.6
	8	50	48.8	38.7	8.16	4.08
Lygus pretensis	9	100	11.3	14.7	34.6	39.4
	10	100	9.6	18.3	31.8	40.3
	11	100	10.8	16.5	29.1	43.6
Pyrellia serena	12	32	6.4	9.6	32.4	51.6
	13	87	3.6	10.6	34.5	48.7

The carpenter ants were observed in the same type of cage, but they were allowed to select from three pieces of an insect-tunneled log. The chunks of wood were dried and then allowed to soak in water for different periods of time. The average of the results of two tests showed that the ants aggregated in wood having a moisture content of from 263.8 to 361.2 per cent; on the contrary, those having from 80.1 to 98.6 to 159.3 per cent water were not chosen.

With the exception of the box-elder bug, *Leptocoris trivittatus*, all of the species exhibited a marked preference for moist situations. There is evidence in the experiments with *Ceratomegilla fuscilabris* and *Hippodamia convergens* that some species, at least may select areas which are neither too wet nor too dry. Both of these species were recovered in moderately wet soil having a moisture content of 20 per cent. An inspection of Table 5 will show that these species were found in places which were similar with respect to the available moisture. In spite of the fact that box-elder bugs are very sensitive to moisture deficiency they seemed to prefer dry leaves and soil and were found most commonly under the same condition in the field.

It was possible to observe the actions of the animals while the temperature was falling in the experimental chamber. All of the species except *Cerato-*

megilla fuscilabris were scattered over the top and sides of the cage at the beginning of each experiment. The latter species was most active on the leaves although a few individuals occasionally ventured up the sides of the cage. Running notes were kept during the time the temperature was falling. It was observed that temperature of about 15°C. initiated the downward movement of both *Leptocoris trivittatus* and *Ceratomegilla fuscilabris*.

Weiss (1913) and Park (1930) have discussed the nature of the stimulus which causes *Ceratomegilla fuscilabris* to descend to the forest floor. They both concluded that a fall in temperature caused the beetles to become negatively phototropic. Park observed the change in photic response at 10°C. Weiss believes, in addition, that with a lowering of the temperature the beetles become very positively thigmotropic. In two experiments similar to those shown in Table 6 the beetles were exposed to complete darkness as the temperature was lowered. The downward movement occurred as it had in the light, and the final distribution of the insects was essentially the same. It seems probably that a geotropic response is also involved.

In the preference experiments many individuals of all the species continued their movement through the leaves and over the soil, even when the temperature was near 0°C. The significance of the observation lies in the fact that those species which do not enter a period of complete dormancy may make changes in their situation even when freezing temperatures occur. The importance of the latent heat of water may be realized in such a circumstance because it would prevent a too rapid sub-zero drop in temperature. An experiment has been designed to demonstrate the effect of soil moisture on the change in soil temperature. Samples of loam having moisture contents of 5, 10 and 15 per cent were placed in small wooden boxes, six inches square. Thermocouples were placed in the soil of each box in such a way that they rested three inches from the top, bottom and sides. The boxes were left uncovered and were placed in a chamber where a constant temperature of —11°C. was maintained. The results of this experiment are shown in Figure 1.

The temperature of the driest soil fell most rapidly to that of the chamber. The temperatures of the other soils were lowered to a point near 0°C., where they lagged for a while before another drop occurred. The effect of the latent heat of water is most apparent in the curve for soil having a moisture content of 15 per cent. The temperature remained at zero for about two hours, even with the small amount of soil and water used in the experiment. The effect of moisture may be very important at the time when free water is in the process of freezing; this is also the time when some species of insects are still searching for shelter.

THE RESISTANCE TO DESICCATION OF SOME INSECTS TAKEN FROM DIFFERENT HIBERNACULA

It has already been shown in a previous section that many insect species are well protected from the danger of excessive evaporation of water from their tissues. There are, however, species which are more or less exposed to the atmosphere and which might occasionally suffer from desiccation during the winter. For the purpose of this study some of both types of insects were collected during the fall and early winter. The following species are usually found in situations where the atmosphere is saturated with moistures: *Phyllophaga fusca* and *P. rugosa, Leptinotarsa decemlineata, Lucilia serricata,* and *Ceratomegilla fuscilabris.* The species which were used

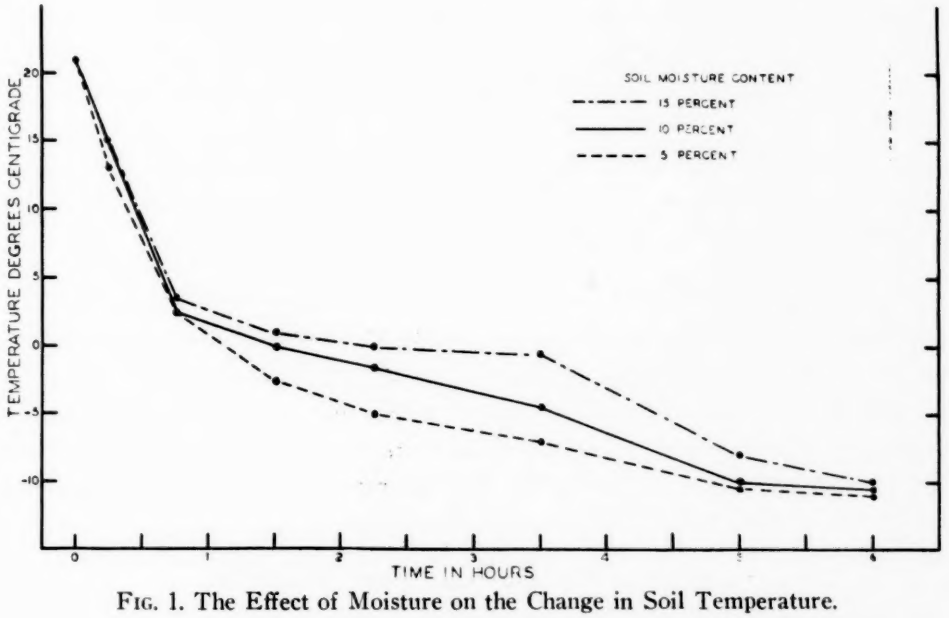

FIG. 1. The Effect of Moisture on the Change in Soil Temperature.

to represent types occasionally exposed to drying were: *Hippodamia convergens, Polistes variatus,* and *Leptocoris trivittatus.* Ten or more individuals of each of these species except *Lucilia serricata,* were handled separately during the desiccation process. Each insect was placed in a small cylindrical wire cage, and then each species group was exposed to a relative humidity of 20 per cent in a glass desiccator. The relative humidity was maintained by means of a sulphuric acid solution prepared according to the tables of Wilson (1921). The insects were weighed every four days and examined every two days to determine mortality. All of the tests were run at 2°C. in order to induce a state of dormancy in the insects. The problem of ventilation was avoided by the frequent examinations and the quiescent condition of the insects at 2°C. The sulphuric acid solutions were replaced every week with fresh material to provide a uniform atmosphere through-

out the period of study. At the beginning of each experiment the initial weight of the animals was determined to the fourth place on a chemical balance. In addition, the percentage of water and dry matter of a sample of individuals from the same collection was calculated. The dry weight of the insects was determined by heating them for 24 hours at a temperature of 105°C. During the course of each experiment the dead individuals were removed as soon as they were observed. They were weighed and their dry weight was determined. The weighing of all individuals, either dead or alive, was accomplished as rapidly as possible because of the rapid evaporation in the low humidity and high temperature of the laboratory. The insects were weighed in a small, closed weighing bottle, and the living ones were returned to the cool desiccator immediately. Some error may be expected from the loss of weight during the weighing. Each apparently dead individual was examined under a binocular microscope for any voluntary movements. This examination reduced the error in the final calculated water content to a minimum.

In treating the results of this study a description of the condition of each species will be given, with remarks about the time and place in which they were collected.

Experiment No. 1. *Leptocoris trivittatus* A

A collection of box-elder bugs was made November 7, 1934, at University Farm, St. Paul. The insects had concealed themselves under a pile of boards and trash near the foundation of a building. In this situation they were protected from rain and their immediate environment was dry. They were very active when warmed in the laboratory but their flattened abdomens made them appear to be in poor condition.

Experiment No. 2. *Leptocoris trivittatus* B

These bugs were collected February 2, 1936, at Battle Creek Park, St. Paul. They were found in the crevices of the walls of a shallow sandstone cave. Several hundred insects were collected at this time and they were in very good condition. They had the general appearance of bugs which had only recently finished feeding, although they had been in hibernation for over three months. The walls of the cave were moist even though the sandstone holds very little water.

Experiment No. 3. *Phyllophaga* larvae

These larvae, which were probably *Phyllophaga fusca,* were collected in sodland not far from University Farm, St. Paul. The majority of the grubs were found at a depth of 20 inches where the soil moisture content was 16.7 per cent. They were collected November 15, 1934.

Experiment No. 4. *Phyllophaga* adults

The adults were taken at the same time and place as the larvae which were previously mentioned. Three species were included in the collection but only two will be considered here. The species were determined by D. J. Pletch. Eight of the individuals were *P. fusca* and two, *P. rugosa.*

Experiment No. 5. *Leptinotarsa decemlineata*

Hibernating adults of this species were removed from a soil hibernation cage November 8, 1934. The vigor of individuals of this species can be determined by the coloration and luster of the cuticula. The specimens chosen for study were all active and well colored. The soil from which they were taken was a sandy loam with a moisture content of 18.2 per cent.

Experiment No. 6. *Hippodamia convergens*

Large aggregations of this species were not found during the period of field investigation for this series of experiments. In most cases single individuals or groups of two or three were discovered. The material used in this experiment was collected from an aggregation of about 25 beetles. They were taken from under some loose bark of a box-elder tree where their immediate environment was dry. The collection was made at Fort Snelling, January 2, 1935.

Experiment No. 7. *Ceratomegilla fuscilabris*

These beetles were found in large numbers on the surface of the ground, under leaves and grass. They were taken at the same time and place as the previous species, *Hippodamia convergens.* Many of them had chosen depressions in the ground where they were well protected from drying by an abundance of wet leaves. The beetles were very active when warmed.

Experiment No. 8. *Polistes variatus*

Polistes queens were found hibernating by the hundreds in the same cave which was mentioned in connection with Experiment No. 2. The wasps were wedged into cracks in the soft sandstone so tightly that one could extract as many as 50 by pulling on the legs of one. These wasps were collected January 12, 1935. At this time only about 2 per cent of them had died.

An inspection of Table 7 shows at once that all of the groups were very constant with regard to the initial percentage of water contained in the individuals of each group. The uniformity in water content is particularly noticeable in the case of *Hippodamia convergens,* where there was a variation of from 49.7 to 51.6 per cent, and of the white grubs which varied only from 83.6 to 87.3 per cent. This uniformity persists in spite of the fact that there are sometimes considerable differences in weight. With regard to this condition, it is interesting to compare the conclusions of Buxton

(1930). He remarked that it is impossible to produce a standard meal-worm with respect to the percentage of water. There is actually a better agreement in water content among the hibernating individuals used in this experiment than among the meal-worms which he reared in carefully controlled cultures.

The differences in the percentage of water in the various species when they were removed from hibernation is not significant because of the great variation in the size of the species and the amount of cuticula. The great difference in the proportion of water in the *Phyllophaga* larvae and *Hippodamia convergens* may have no relation to the problem of water balance. There may be a very similar condition within the tissues of the two species. Buxton (1932) had discussed this question with regard to changes in the proportion of water during the growth of an insect and the proportion of water in insects of different sizes.

The loss of weight in the seven species can be considered a loss of water with the exception of the loss in *Polistes*. Table 7 also shows the relation between the proportion of dry matter in a sample of individuals taken at the beginning of each experiment, and the proportion of dry matter calculated from the dry weight and initial weight of the experimental animals. There is no significant difference in the percentage of dry matter except in the cases of *Polistes,* and *Ceratomegilla*. The animals are protected by low temperatures against the excessive use of reserve fat and their chief loss in weight is water. Rau (1930) has suggested that *Polistes annularis* is favored by a cold winter because the insects remain torpid instead of spending their energy.

The actual resistance to desiccation indicated in Table 7 shows that there is relatively little difference in the time required to kill five of the seven species. *Leptinotarsa decemlineata* was very obviously the most resistant, and the coccinellid beetle, *Ceratomegilla fuscilabris,* was the least resistant. Neither the final water content nor the percentage loss of weight can be used as a measure of resistance. The final water content, like the initial proportion of water, is too dependent upon the size of the species and the amount of scleritization. The percentage loss in weight of the several species cannot be used because it does not take into consideration the rate of loss and the relation between loss and total water available for evaporation. Table 8 shows that the difference in the resistance of individuals of the same species is very definitely more dependent upon their ability to conserve moisture that it is upon their lower limits of tolerance. The per cent variability between the members of each group is given for each of the important variables. In all cases the individual variation in the proportion of water at death is very small, although there is a great variability in the rate in which water was lost as expressed by the total loss in weight and the survival time in days.

TABLE 7. Duration of Life and Loss in Weight of Several Insects at 2°C. and 20 per cent Relative Humidity.

Species	Number Ind.	Initial weight in milligrams	Initial water content in per cent	Final water content in per cent	Total loss of weight in per cent	Survival time in days	Ratio of actual to calculated initial dry matter
Leptocoris trivittatus A.	10	39.1± 3.17*	73.1 ±0.79	52.7 ±1.05	42.6±2.15	28.8±1.93	1.03
Leptocoris trivittatus B.	10	32.2± 2.97	66.9 ±0.85	50.7 ±1.85	32.5±1.62	36.6±2.17	1.02
Phyllophaga larvae	9	113.7± 9.53	84.7 ±0.93	67.5 ±0.98	52.7±0.80	35.8±6.68	1.07
Phyllophaga adults	10	581.2±30.49	71.27±0.92	53.95±0.91	38.0±1.20	35.2±1.84	1.01
Leptinotarsa decemlineata	9	172.9± 7.63	69.2 ±1.06	49.7 ±1.87	38.6±1.89	57.3±8.74	1.01
Hippodania convergens	7	21.3± 1.01	51.0 ±0.33	36.1 ±0.97	24.4±0.71	36.6±4.89	1.01
Ceratomegilla fuscilabris	14	13.1+ 0.47	59.0 ±0.65	44.2 ±1.14	26.5±0.98	23.0±1.79	1.13
Polistes variatus	10	148.5± 8.73	60.4 ±1.19	49.7 ±0.58	20.9±1.17	35.4±2.88	1.14

*Standard error.

As a result of this study it can be seen that there is no relation between the usual type of hibernaculum, with respect to moisture, and the ability to resist desiccation. The most resistant, *Leptinotarsa,* is also one which hibernates in the soil where it would not suffer from desiccation. The least resistant, *Ceratomegilla,* is usually found in wet places and, of course, would be favored by its location. Another species, *Lucilia serricata,* was dried under similar conditions and after five months there was a mortality of only 12.2 per cent. This species lost approximately 50 per cent of its weight during the process. This insect, however, is able to enter a diapause and endure as long as eight months of dormancy. Cousin (1933) has studied *L. serricata* very extensively and has observed this same phenomenon.

Urech (1890) showed that the loss in weight of pupae of *Gastropacha neustria* was influenced considerably by the presence of a cocoon. He exposed pupae for five days over calcium chloride; the naked pupae lost 9.42 per cent weight and those in cocoons lost 3.53 per cent of their weight. A series of larch sawfly larvae *(Lygaeonematus erichsonii)* were exposed for 16 days to a relative humidity of 20 per cent at a temperature of 2°C. Naked larvae lost 7.79 ± 0.48 per cent weight and those in cocoons only 2.65 ± 0.006 per cent weight. The larvae within the cocoons actually lost

TABLE 8. A Comparison of the Coefficients of Variability of Some of the Variables Associated with Resistance to Desiccation.

Species	Initial weight in milligrams	Initial water content in per cent	Final water content in per cent	Total loss of weight in per cent	Survival time in days
Leptocoris trivittatus A.	24.36	3.21	5.84	15.16	20.14
Leptocoris trivittatus B.	27.73	3.80	10.92	15.08	17.84
Phyllophaga larvae	23.61	3.09	4.13	4.28	52.70
Phyllophaga adults	15.74	3.89	5.06	9.52	15.39
Leptinotarsa decemlineata	12.47	4.35	10.66	13.88	43.21
Hippodamia convergens	11.59	1.61	6.57	7.05	32.78
Ceratomegilla fuscilabris	13.24	3.96	9.32	13.38	28.17
Polistes variatus	15.62	5.9	16.8	24.4	15.62

no water. The loss in weight was entirely from the cocoon itself. A discussion of the relation of these cocoons to the larvae will be included in a later section.

Four experiments were performed with *Polistes variatus* and *Leptocoris trivittatus* to determine the nature of the loss of weight in a saturated atmosphere. Individuals of these two species were collected at Battle Creek Park, St. Paul, at the same time as the previously mentioned visit was made there. The experimental animals were placed in small wire screen cages over distilled water in glass desiccators. Weighings were made every four days and the dead individuals removed at these times. Because of a time limitation it was not possible to carry these experiments to the point where all the individuals had died. There was a slightly greater loss in water from the box-elder bugs which were kept at 6°C. than from those exposed to 2°C., 16.1 ± 1.36 and 13.75 ± 0.93 per cent respectively. A comparison of the proportion of dry material in the experimental animals with that of a sample taken at the beginning of the experiment again shows no essential difference. There was a rather rapid rate of mortality among the bugs kept at 6°C., and the water content at the time of death was higher than when similar insects were exposed to dry air. It is evident that there is more than a minimum water requirement which must be satisfied for survival.

The wasps showed a much greater difference in their response to the two temperatures. At 6°C. the percentage of weight lost, 16.2 per cent, was very similar to that of the box-elder bugs, but at 2°C. the loss of weight, 5.9 per cent, was much less. However, the loss in weight was not entirely water. The percentage of dry matter in the individuals exposed at 6°C. was less than that of those exposed to 2°C. There was a very appreciable loss of material other than water. This loss was expected because the insects became very active almost immediately after they were placed at room temperature. They were not completely dormant, but those kept dry or in a saturated atmosphere at 2°C. remained quiet for a considerable length of time after being placed at room temperature. In spite of a loss of dry weight and of water, only one wasp died during the 44 days in which the experiment was conducted.

During the winter of 1934-1935, a series of collections was made periodically to determine the margin of safety between the water content of hibernating species and the lower limits of resistance which were demonstrated by desiccation in the laboratory. Some of the species are those which have just been discussed above; others were not studied as individuals but were dried as a group. The dead insects of the latter species, however, were weighed and oven-dried on the day when the observation of death was made. The list of species, their proportion of water at death, their lowest proportion of water during hibernation, and the type of hibernacuum will be found in Table 9.

With the exception of *Leptocoris trivittatus, Hippodamia convergens, Polistes variatus* and *Lygus pretensis,* none of the species tend to lose enough water to approach the lethal limit. Those four species do show, however, that even during the winter when the relative humidity is comparatively high and the temperature low, some species may suffer from the excessive loss of water.

TABLE 9. A Comparison Between the Proportion of Water at Death and the Lowest Water Content Observed During the Winter.

Species	Per cent water at death	Winter low water content	Hibernacula
Leptocoris trivittatus	50.7	57.4	In crevices of sandstone cave
Leptocoris trivittatus	50.7	56.8	Under boards and trash
Leptocoris trivittatus	50.7	62.4	In wet leaves, on ground
Polistes variatus	49.7	56.5	In crevices of sandstone cave
Hippodamia convergens	36.1	42.4	Under leaves, on surface of soil
Hippodamia convergens	36.1	43.7	Under loose bark
Ceratomegilla fuscilabris	44.2	58.2	Under leaves and grass
Camponotus herculeanus pennsylvanicus	49.6	59.7	In oak log
Pyrellia serena	58.9	69.7	In badly decayed log, very wet
Lucilia serricata	51.0	66.9	In soil
Amblyteles sp.	46.3	58.7	Under bark of decayed log
Vespula maculata	47.3	61.4	On soil, under logs
Phyllophaga fusca (adults)	54.2	68.9	Deep in soil
Lygus pretensis	44.3	52.8	In sod, or base of grass stems
Carpocapsa pomonella	55.9	65.4	Under bark of apple trees, protected by cocoon

THE EFFECTS OF ENVIRONMENTAL MOISTURE ON SURVIVAL AT LOW TEMPERATURES

When it became apparent that many species endured a wet environment during the winter, a series of experiments was planned to determine the effects of environmental moisture on the survival of insects at winter temperatures. The experimental animals used in this study were as follows: *Leptocoris trivittatus, Ceratomegilla fuscilabris, Polistes variatus, Lucilia serricata,* and *Cynomyia cadaverina.* The adults of the first two species and prepupae of the last two were employed. When the effects of relative humidity were studied, the insects were caged in small, screen-covered pill boxes; these containers were placed in desiccators. The insects which were exposed to various temperatures in soil or leaves were likewise confined in similar pill boxes. The soil and leaves were oven-dried, and then the desired amounts of water were added. The mortality of the insects was recorded at the end of each period of conditioning, and then again after the animals had been exposed to a lethal low temperature. The undercooling points and observed freezing points, which were determined in some of the experiments, were obtained in two ways. The readings for *Lucilia serricata* were taken with a copper-constantan thermocouple and a Leeds-Northrup Potentiometer, after the method of Robinson (1928a). The freezing points of *Polistes* were taken with a thermocouple system devised by Salt (1937). For the purpose of ob-

taining the freezing points of *Lucilia*, a Dewar flask was fitted with a copper expansion coil which was connected with a tank of liquid carbon dioxide. A nearly constant temperature could be maintained in the flask by the judicial use of a needle valve. The blowfly larvae were exposed to a temperature of —25°C. when they were to be frozen.

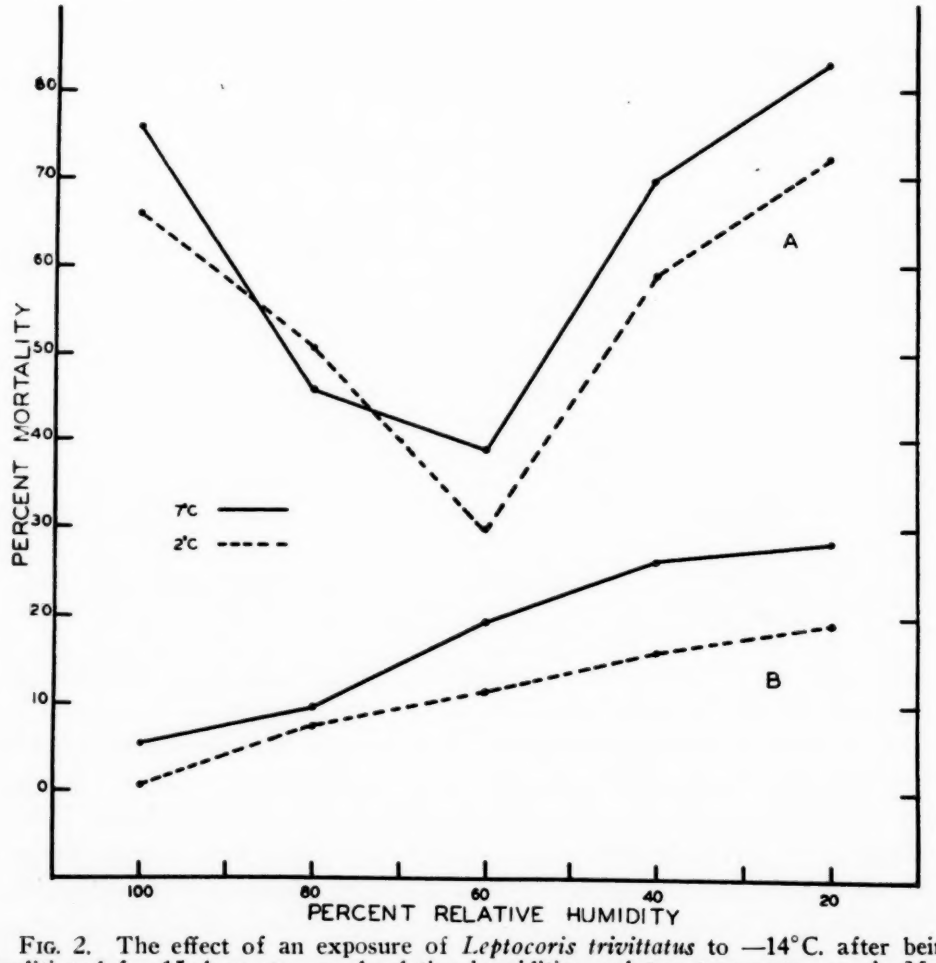

FIG. 2. The effect of an exposure of *Leptocoris trivittatus* to —14°C. after being conditioned for 15 days at several relative humidities and two temperatures. A. Mortality at —14°C. B. Per cent loss in weight during conditioning.

Box-elder bugs were collected in the early winter from caves at Battle Creek, St. Paul. Groups of 100 indivdiuals were conditioned at 2°C. and 7°C. and five different relative humidities for periods of 15 and 30 days. It is apparent from Figure 2 that while the box-elder bugs are exposed to moderately low temperatures, their chances for survival decrease as the atmospheric moisture decreases. The effect of different temperatures is to alter the saturation deficiency when the relative humidity is held constant. This action can be seen in the results at 7°C. and 2°C. There is a greater loss of weight and a correspondingly higher mortality at 7°C. when the time of ex-

posure is the same at both temperatures. Figure 2 shows the effect of the loss of weight on survival at a low temperature of —14°C. The bugs were exposed at this temperature for eight hours. There is an increase in the resistance to a temperature of —14°C. as the relative humidity is decreased. However, the increase in resistance proceeds to a relative humidity of only 60 per cent. In drier air the animals are killed much more easily after being exposed at either of the conditioning temperatures. The effect of the rate of loss of water is not entirely responsible for the increased resistance. Other factors such as the relation of free and bound water may be important. It is obvious that the conclusions of Payne (1929), with regard to the relation of winter hardiness to absolute humidity, are rather limited. Longer exposures to some of the temperature-humidity combinations or lower humidities probably would not have produced the same effect. Salt (1937) concluded that desiccation did not affect the hardiness of this species. He tested cold resistance by measuring the undercooling point. The use of this method involved a much shorter exposure to low temperatures and for this reason the results are not comparable with those just discussed.

TABLE 10. Effect of Soil Moisture at Low Temperatures on *Ceratomegilla fuscilabris.*

Per cent soil moisture	Per cent mortality at 2°C.	Per cent mortality at —3°C.	Per cent mortality at —6°C.
40	0	82.7	21.2
30	0	76.6	16.6
20	3.3	33.3	10.3
10	3.9	63.4	14.3
0	100.0	89.0	....

In addition to the atmospheric moisture which surrounds insects while they are in hibernation, there may be effects produced by contact with other moist media, such as soil, wood, etc. A few experiments have been designed to demonstrate these effects. An aggregation of the beetles, *Ceratomegilla fuscilabris,* was collected early in the fall. Groups of 30 beetles each were placed in pill boxes containing loam with the following different percentages of moisture: 40, 30, 20, 10 and 0. One lot was conditioned at 2°C. for one month and then exposed for one week at 6°C. —6°C. The other lot was exposed at —3°C. for one month. The results of these experiments will be found in Table 10.

The effects of moisture on these beetles were quite similar to those which were determined for the box-elder bugs. A wet environment was most favorable for survival at temperatures above freezing; a moderately wet environment was the most desirable at sub-zero temperatures; a dry environment was unsuitable at all times. When these beetles were allowed to make a moisture selection as they were in the experiments which were reported above, the majority of them congregated on soil having a moisture content of about 20 per

cent water. Conditions associated with soil of this same moisture content were the most favorable at the temperatures studied.

Another series of experiments has shown that the prepupae of the blowfly, *Cynomyia cadaverina*, are favored at low temperatures by having been conditioned in either sand or loam with a low moisture content, but they are better able to endure a temperature just above freezing when they have been in moist soil. The prepupae were exposed for two weeks in both sand and loam at 2°C., and after being removed from the soil, were subjected to a temperature of —10°C. for eight hours. The moisture content of the soil media and the mortality records are included in Figure 3. Approximately 200 prepupae were used in each soil container.

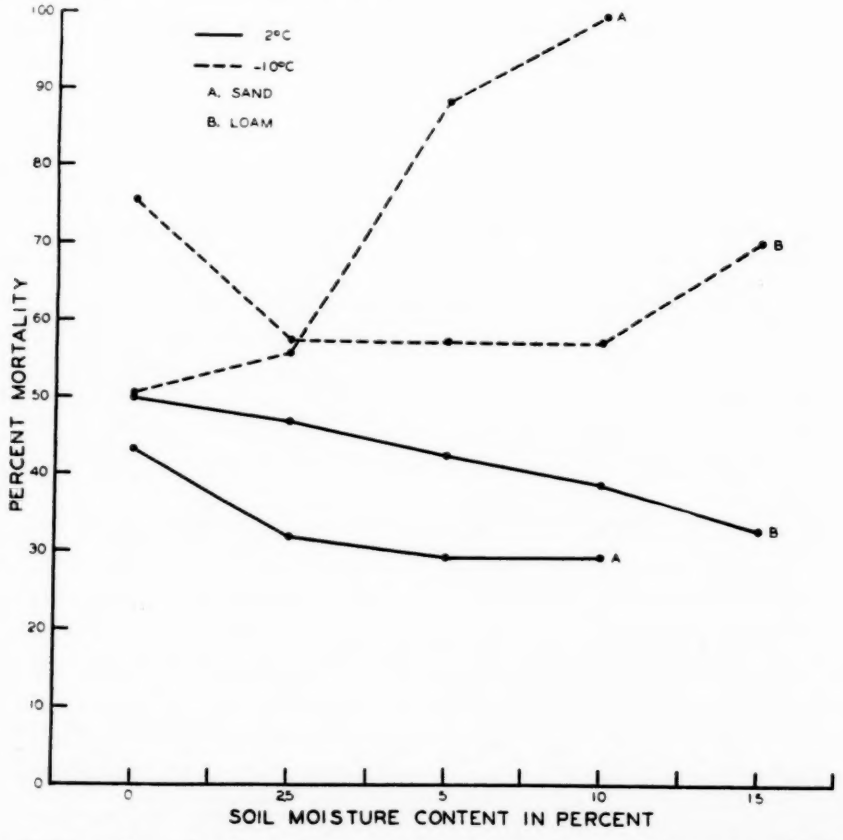

FIG. 3. The effect of soil moisture on the cold hardiness of *Cynomyia cadaverina*.

It will be observed in Figure 3 that there is a progressive decrease in hardiness as the water content of the sand increases. In the loam there was first an increase in hardiness and then a decrease as the soil water content rose. Because of the hygroscopic nature of these two soils, there is a difference in the amount of water available for evaporation. The hygroscopic coefficients of the sand and loam were found to be 0.38 and 8.9 per cent, respectively. This difference will account for the high mortality of the prepupae at

2°C. in loam, and the low mortality at —10°C. when they were conditioned in the same material. A partial desiccation was favorable for cold-hardiness, but a more severe desiccation in dry loam was unfavorable.

During the winter of 1934, a series of experiments was performed to determine the effect of relative humidity on the freezing and undercooling temperatures of *Lucilia serricata*. From the start it was very apparent that the amount of variation among the individuals having had the same treatment was too great. The undercooling temperatures varied from —5°C. to —22°C., and the observed freezing points varied from —1.5°C. to —13°C. When handled these larvae became very moist. Many which had not been handled were

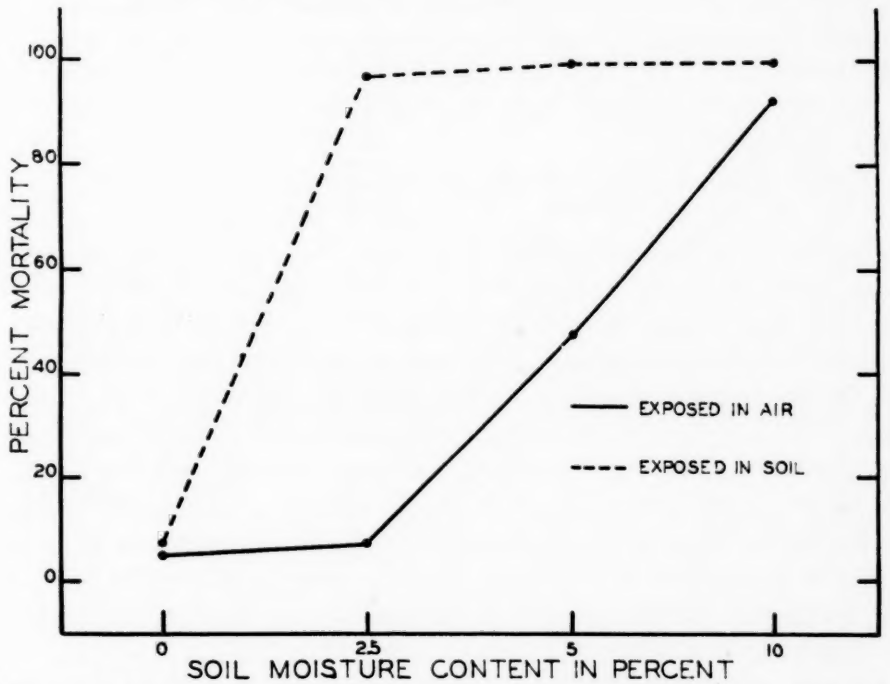

FIG. 4. The effect of exposing prepupae of *Cynomyia cadaverina* to —10°C. in air and sand.

wet because of the condensation of atmospherice moisture on their bodies. At first the possible effect of contact moisture on the undercooling and freezing points was not considered. However, at the same time a series of the prepupae of *Cynomyia* was conditioned as in the previous experiment except that they were allowed to empty their digestive tracts completely before being placed at low temperatures. These prepupae were exposed for eight hours at a temperature of —10°C. They were placed in sand having the same moisture content as that in which they had been conditioned. In order to put the larvae down in the sand in the same position in all the containers, they were enclosed in sand-filled, wire cages which were all buried to the same depth in coffee tins. The results of this experiment as shown in Figure 4 were quite unexpected.

The prepupae which were exposed directly to the air behaved essentially the same as those described in Figure 4. They were more resistant than the larvae used in the previous experiment because they had been allowed to finish their feeding before the conditioning process was begun. The mortality of the larvae buried in dry sand was very little different from the mortality of similarly dry larvae exposed to the same temperature in air. The unexpected high mortality of larvae treated in sand having moisture contents of 2.5, 5 and 10 per cent, as shown in Figure 4 was difficult to explain. At first it was thought that the body fluids of the insects might have been diluted by the free water in the sand. However, the larvae which had been left in open tins during the freezing experiment had previously been conditioned in sand having like percentages of water. They were weighed before and after freezing. There was a very small loss in weight during the treatment, but it was not sufficient to have caused the great difference in the resistance of the two groups of larvae.

Another solution was suggested by the fact that there was practically the same rate of killing in the three sand preparations which had been moistened. The hygroscopic coefficient of this sand had been found to be .83 per cent. Consequently, even the sand, having a moisture content of only 2.5 per cent, contained more water than could be absorbed on the particles. These observations led to the conclusion that the presence of free water in contact with the larvae could inoculate the tissues of the insects when the water was frozen. Harvey (1918) had observed the same phenomenon in the case of some plants which were not protected by a waxy coating over the leaves. In such plants, water is able to adhere to the surface of the leaves in droplets which may be deposited by condensation from the surrounding atmosphere. Harvey remarks, "Water which freezes on the leaf surface serves to inoculate the undercooled solution within the leaf; in fact the injected spots observed are caused by this inoculation."

Other experiments have been carried out to test the value of this theory of the action of contact moisture. Prepupae of *Lucilia sericata* were exposed in sand and loam at several low temperatures. The moisture contents of the sand were 0, 2.5, 5, 10 and 15 per cent; those of the loam were 0, 5, 10, 15, 20, 25 and 30 per cent. In all cases a check sample of 100 larvae was exposed in the air at the same temperatures. The sand and loam were taken from stock mixtures in small quantities for this experiment. The amounts used were of a sufficient quantity to provide good contact with the larvae but not enough to cause a very great lag in the fall in temperature. Table 11 shows the results of cooling prepupae for five hours in sand.

With this species there is also a marked effect of contact water on resistance. In this case all the larvae were treated alike before they were placed at —12°C. and —15°C. The difference in the mortality at the two temperatures is probably due to the fact that —12°C. is very close to the average freezing

TABLE 11. The Per Cent Mortality of *Lucila serricata* Exposed to Low Temperatures in Wet Sand.

Temperature	Air	Per cent age moisture content of sand				
		0	2.5	5	10	15
—12°C.	8	7	82	93	96	100
—15	32	30	100	98	100	100

point of undisturbed individuals, as determined by the undercooling method. At —15°C. the effect of the contact moisture is again very evident as can be seen in the mortality of prepupae in all the sand preparations which contain water.

It has been shown that sand, with a low water holding capacity, contained enough water at low soil moisture percentages to have an influence on the resistance of *Lucilia* prepupae to cold. Another experiment with the same insects substantiates the theory that the available water in the soil is very important. Prepupae were exposed in loam which had a hygroscopic coefficient of 8.9 per cent. The water content of the soil samples and the experimental temperatures are included in Table 12 with the mortality records at the various combinations of temperature and moisture.

TABLE 12. The Percentage Mortality of *Lucilis serricata* Exposed to Low Temperatures in Wet Loam.

Temperature	Air	Per cent moisture content of loan						
		0	5	10	15	20	25	30
— 6°C.	0	0	0	2	5	4	0	3
— 9	2	4	3	50	54	52	54	56
—12	10	4	7	86	98	96	97	100
—15	36	33	41	92	100	100	99	100
—18	98	94	96	100	100	100	100	100

One hundred prepupae were treated at each of the several combinations of temperature and moisture. They were exposed for a period of five hours. Table 12 and Figure 5 show the final results. When the larvae were cooled in soil having moisture contents less than 10 per cent, there was no essential difference between the mortality in the soil and the mortality in the air. At 10 per cent and above there was a very high mortality at temperatures lower than —10°C. Two points of importance are emphasized by Figure 5.

First, the soil moisture does not affect the resistance to low temperatures until it is present in quantities greater than that which can be absorbed by the soil. Secondly, there is a definite temperature zone in which the action of soil moisture had the greatest effect upon the mortality caused by freezing. This range of temperature is believed to be associated with the normal freezing point of the species.

Because of the difficulty in obtaining a series of freezing point determinations for the *Lucilia* prepupae, another species has been investigated further. *Polistes* wasps were placed between layers of moistened cheese cloth and then exposed to several low temperatures for five hours. The undercooling and observed freezing points of other individuals were taken when the wasps were both wet and dry. Figure 6 gives additional evidence to support the belief that contact moisture may be of importance in the natural cold resistance of insects.

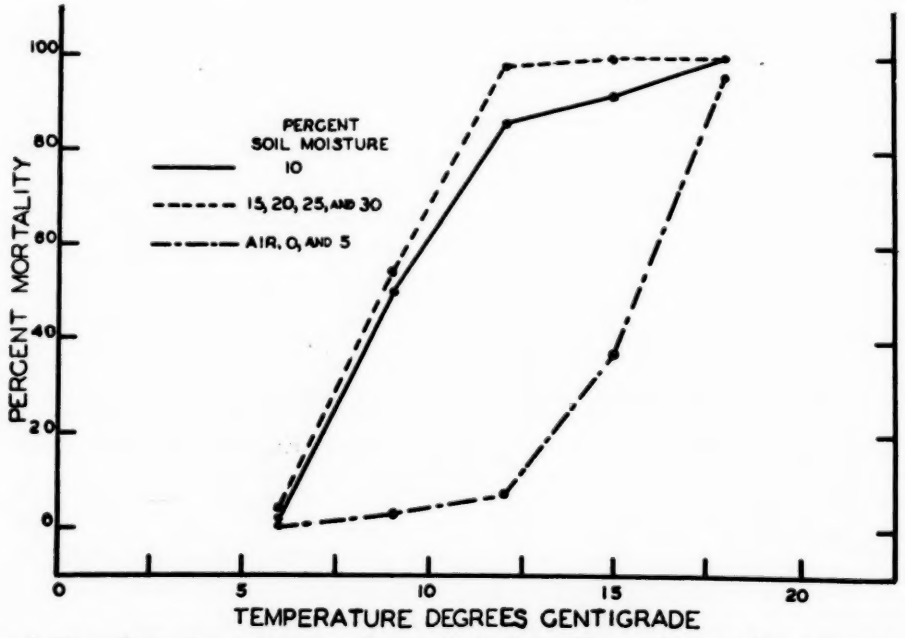

FIG. 5. The effect of wet loam on the survival of *Lucilia serricata* at low temperatures.

When 100 wasps, both wet and dry, were frozen, the mortality curves were very similar to those constructed previously for the blowfly larvae. It is again evident in Figure 6 that contact water may increase the rate of mortality when insects are exposed to low temperatures. However, the facts presented so far do not explain the manner in which the influence of contact water is exerted. Two suggestions have already been proposed, namely: contact water may hydrate the insects' tissues and consequently raise the freezing point; or the water which freezes on the surface of the animal's body may inoculate the tissue within, thereby preventing the undercooling process. Other experiments have been performed in the hope of clarifying this question.

The individual undercooling and observed freezing points of several wasps have been determined electrometrically according to the method of Salt (1937). Both dry wasps and individuals which had been wet were frozen. The moistening of the insects was accomplished by submerging them quickly into a beaker of water, after which they were shaken to remove any large

drops and then fastened firmly against the thermocouple junction. In the case of a few individuals which were not submerged, a drop of water was placed on the dorsal surface where it would not come in contact with the thermocouple. The undercooling and observed freezing temperatures of several dry and wetted wasps are listed in Table 13. With the exception of three individuals, all of the dry wasps showed typical undercooling and freezing, and they were dead when removed from the low temperature cabinet. The three which lived did not show typical rebounds, the undercooling points were high, and the rebounds were small. The average observed freezing point of the other 13 insects was 13.1°C.

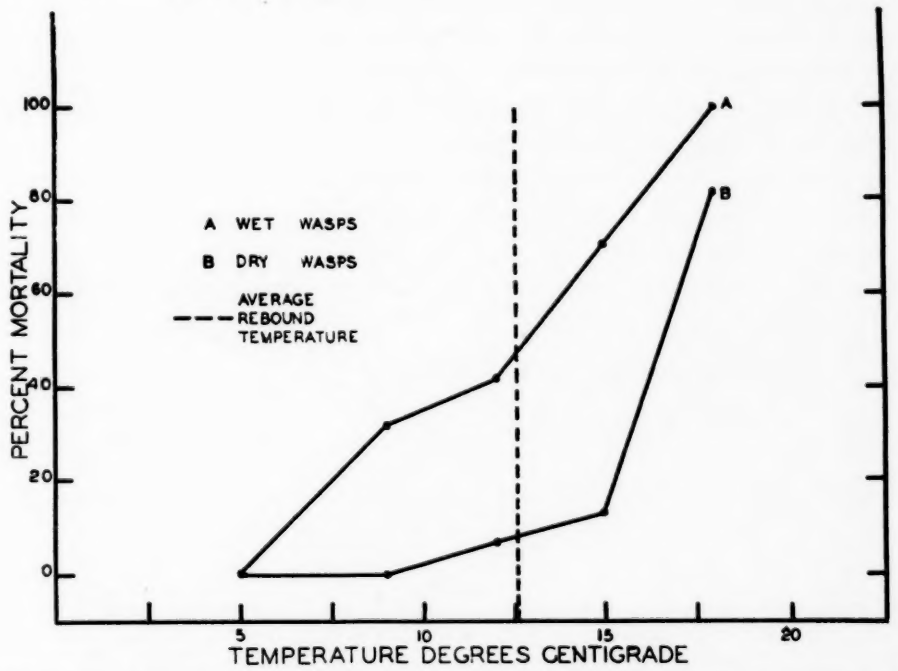

FIG. 6. The effect of contact moisture on the survival of *Polistes* wasps at low temperatures.

The temperature readings taken for the wet wasps are very different from the dry wasps which have just been considered. There was no undercooling of the insects. The high undercooling temperature and small rebounds indicated in Table 13 for this group represents the freezing of water on the surface of the insects' bodies. In most cases there were two or more small rebounds instead of a single one. The temperature limits of the largest of the rebounds are included in the table. It will be noticed that four individuals did not exhibit any rebound. These four insects had had water applied only to the dorsal surface. The small amount of heat produced by the freezing of this water was unable to affect the thermocouple which was under the thorax. Time-temperature curves for dry wasps, wasps which had been

dipped in water, and those which had been moistened only on the dorsal surface are shown in Figure 7.

The time was taken with a stop-watch while the temperature was read continuously from a calibrated galvanometer scale. Curves D and E show the typical undercooling and freezing of normal individuals. Curves A and B are those of two wasps which had been dipped in water. The multiple rebounds can be seen in the upper portion of the temperature scale. These disturbances are probably caused by the freezing of isolated drops of water which were present on the legs or other parts of the ventral side of the body. It will be noticed that there are no rebounds comparable to those seen in curves D and E. Curve C illustrates the manner in which the insects cooled when water was placed only on the dorsal surface. There is a gradual drop in the body temperature with no evidence of a rebound. However, there is a slight lag in the drop in temperature at about —10°C., which may have been caused by the initial freezing of the tissues.

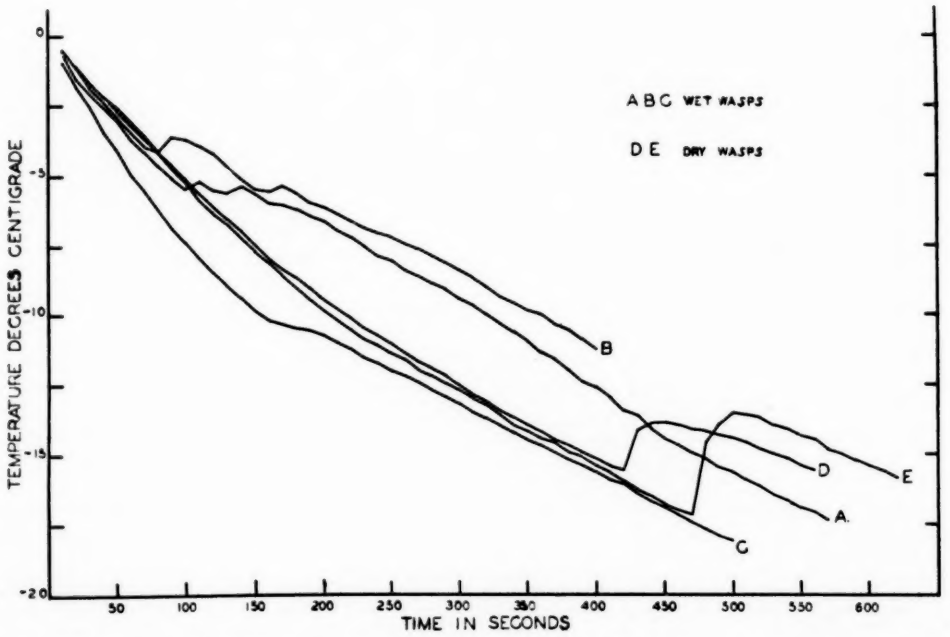

FIG. 7. Time-temperature curves for wet and dry *Polistes* wasps exposed to freezing temperatures.

Two important points have been brought out in the freezing of individual wet insects. First, the presence of water on the surface of the body prevented the insect body fluids from undercooling to a temperature below the normal freezing point. Secondly, although there had been no undercooling, the insects were frozen and dead when removed from the cabinet. The wet insects described in Figure 7 and Table 13 always died if they were allowed to cool to temperatures below the average observed freezing point of the dry wasps.

These observations lead to the conclusion that in all probability the body fluids of the insects were actually inoculated by the freezing of water on the surface of the body. It does not seem possible that the brief submersion could have diluted the body fluids sufficiently to change the salt concentration. However, the inoculation may be associated with the partial hydration of the tissues at the point where contact with surface moisture occurs. The hydrated tissues would freeze at higher temperatures than the rest of the insect and would initiate additional freezing as soon as the temperature was lowered sufficiently. The amount of freezing necessary to cause death has not been determined definitely for the wet wasps. The dry wasps were all removed from the temperature cabinet immediately after the observed freezing temperature was recorded, and they were found to be killed without complete freezing. Wet wasps removed at temperatures near the average freezing point of the dry individuals were also dead. However, a comparison of the mortality curves with the broken line in Figure 7 shows that the effect of contact moisture may be observed at temperatures above the usual freezing point. The apparent inconsistency has now been explained by Salt (1937).

TABLE 13. The Freezing and Undercooling Temperatures of Dry and Wet *Polistes* Wasps.

	Dry Wasps			Wet Wasps		
Number	Undercooling temperature	Freezing temperature	Remarks	Undercooling temperature	Freezing temperature	Remarks
1.....	—17.1°C.	—13.4°C.	dead	—4.8°C.	—4.6°C.	Removed —20°C. dead
2.....	—10.0	— 8.1	living	—4.6	—4.5	Removed at once living
3.....	—16.8	—13.6	dead	no rebound	.....	Removed —20°C. dead
4.....	—15.4	—11.9	dead	—6.1	—5.8	Removed at once living
5.....	—17.3	—14.1	dead	—3.8	—3.7	Removed —15°C. dead
6.....	— 7.2	— 6.9	living	—5.7	—5.2	Removed —15°C. dead
7.....	—18.3	—15.2	dead	no rebound	.....	Removed —22°C. dead
8.....	—17.4	—13.9	dead	—5.3	—5.2	Removed at once living
9.....	—17.6	—12.1	dead	—4.6	—4.1	Removed at once living
10.....	—16.3	—12.6	dead	—4.9	—4.7	Removed at once living
11.....	—15.4	—11.1	dead	—6.1	—5.7	Removed at once —15°C. dead
12.....	—12.2	—11.1	living	no rebound	.....	Removed —22°C. dead
13.....	—16.1	—13.3	dead	—5.4	—5.1	Removed —20°C. dead
14.....	—18.6	—12.8	dead	—6.6	—5.2	Removed —20°C. dead
15.....	—12.8	—13.6	dead	no rebound	.....	Removed —20°C. dead

A question naturally arises with regard to the manner in which the continuity between the body fluids and the surface water is established. Several workers have demonstrated that the cuticula of insects is not impervious to liquids. Eidmann (1922) discovered that the insect cuticula may act as an osmotic membrane. He was able to pass weak acids and alkalis through chitinous membranes dissected from the cockroach. Shafer (1915) concluded from his studies of contact insecticides that many of the powders probably go into solution in exudates at the base of the legs and wings, and then pass through the thin membranes in these locations.

Wilcoxon and Hartzell (1933) proved that aqueous solutions of pyrethrins could penetrate the insect body without entering the tracheae. The solutions were colored with the dyes oil red, Sudan III, and "oil dag." There was a tendency for the stains to appear in the hypodermis along the conjunctivae and below the pores of setae. Bodine (1933) and Slifer (1934) found that water would pass through the chorion of grasshopper eggs when they were submerged in hypotonic salt solutions. Slifer emphasized the fact that the resistance of grasshopper eggs to hydration was due to the relatively impermeable chorion. This may be the reason that Parker (1930) found no difference in the resistance to freezing of grasshopper eggs which had been kept in wet and in dry sand.

Salt (1937) in a critical study of the importance of contact water observed that some insects were not easily inoculated by drops of water on their cuticula. The eggs of *Malacosoma disstria* showed no difference in resistance to freezing whether they were exposed to a low temperature after being soaked in water or dry. The resistance of this species to submergence has been studied by the author. It was found that the chorion was impervious to water unless the caterpillars started to chew their way out of the egg.

THE EFFECT OF CONTACT AND ATMOSPHERIC MOISTURE ON THE ELEVATION OF THE WATER CONTENT OF SOME INSECTS

During the spring of 1934, it was noticed that the water content of hibernating *Leptocoris trivittatus* adults increased when the bugs were in contact with wet leaves. Other bugs exposed to a saturated atmosphere in a cave showed no like increase until the insects had become active and were feeding. These and other observations made it seem desirable to study the manner in which dormant insects take up water from the environment. The following insects were studied for the purpose: prepupae of *Lucilia serricata*, adults of *Leptocoris trivittatus*, adult *Polistes variatus*, and the larvae of the larch sawfly, *Lygaeonematus erichsonii*. When a saturated atmosphere was desired, the insects were held in wire cages over distilled water in desiccators. The contact moisture was supplied by placing the insects in between layers of moistened cheese cloth. The cheese cloth and insects were enclosed in screen covered pill boxes which were also placed over distilled water in desiccators. The insects were weighed before each experiment and again at the end. The body surface was carefully dried before weighing the insects which had been in actual contact with water.

A collection of miscellaneous hibernating box-elder bugs was sorted into three groups according to their general activity. Those which were very active and seemed vigorous were called "active"; those which were able to move about although rather feebly, were considered "fair"; and the individuals

which could move their appendages but could not crawl, were considered "poor." Ten insects from each of the three groups were exposed to contact moisture at 2°C. and at 6°C. for eight days. The percentage gain in weight and other data pertaining to this experiment are found in Table 14. It will be noticed that the average gain in weight is quite different at the two temperatures. The insects treated at 6°C., with the exception of the "poor" individuals, showed a much greater per cent gain than all three groups which were studied at 2°C. This difference is illustrated clearly in Figure 8 which also shows the relation between the initial water balance and the ability of this species to take up water. After the initial water content had been calculated for each of the individuals used at both temperatures, their histograms were constructed. The columns represent these calculated values arranged consecutively from the smallest to the greatest. When the performance of the individual insects was compared in this way it was evident that the initial water content regulated the amount of water which was taken up at 6°C. The quantity of water gained is represented in Figure 8 by the final water content which is expressed as per cent of the total weight. The individuals which had lost enough water by evaporation to approach the lethal limits for the species gained very little weight. Most of them had been included in the original arbitrary group called "poor." With one or two exceptions their activity did not change after having been in contact with water. The "fair" insects, those which had lost less water, showed a very remarkable gain in weight which was accompanied by a marked improvement in their general activity at room temperature. The group which had been called "active" took up more water than the first one discussed, but much less than the second. In most cases these active individuals had an initial water balance which closely approximated the normal percentage for the species. The water content of the individuals represented in the center of the lower histogram of Figure 8, tended to increase to the proportion of about 70 per cent, which is the percentage usually found in feeding insects.

The gain of water at 2°C. was very little in all three arbitrary groups. Section A of Figure 8 shows that the change in the proportion of water had no relation to the initial water content. A series of dry, dead individuals showed about the same gain in weight at either temperature; therefore, it is believed that the water which was taken up at 2°C. was absorbed mostly by the cuticula. The same is probably true for the "poor" individuals studied at 6°C.

Several conclusions can be drawn from this experiment. In the first place, the ability for insects of this species to take up water is regulated by the extent to which desiccation has been carried, and by the temperature. The gain in water is probably not merely an adsorption of water by the tissues but is in some way related to the metabolism of the insects. The possibility of drinking has been eliminated by the quiescent condition of the

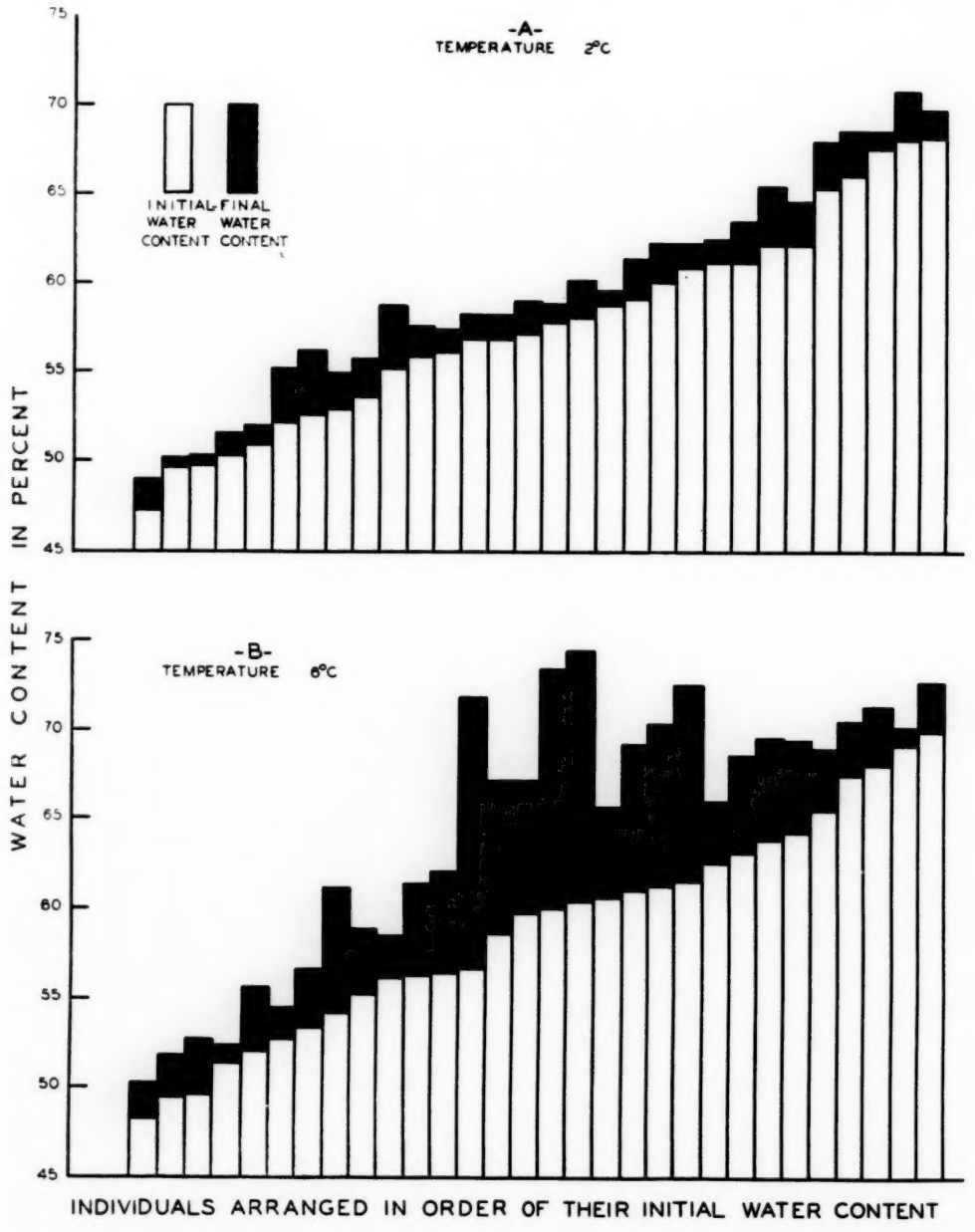

FIG. 8. The relation between the gain in water and the initial water content at different temperatures.

bugs at both temperatures. In the second place, the general activity of these insects is also closely regulated by the water balance. "Activity" is used here in the sense of well-being. In the third place, the insects are protected against the excessive hydration of the tissues by the action of low temperature. A gain in water during the winter would make them more susceptible to cold. Finally, this species, at least, is able to take up water and increase

its ability to move about while it is still in a dormant condition. In order to test the value of these conclusions, additional studies on this species and investigations of some other species have been carried out.

Hibernating *Polistes variatus* queens were exposed to contact moisture in the same manner as were the box-elder bugs discussed in the first experiment of this series. At 2°C. "active" individuals showed a percentage gain in weight of 15.52 ± 3.37 while the "poor individuals gained 4.88 ± 1.45 per cent; at 6°C. the gains were 20.61 ± 2.64 and 4.35 ± 0.34 per cent respectively. The considerable increase in weight of "active" wasps at 2°C. is believed to have been caused by the apparent incomplete dormancy of this species at temperatures above freezing.

Four groups, of eight box-elder bugs each, were weighed and then exposed for five days in dry and wet leaves at 2°C. and 6°C. in a saturated atmosphere. The leaves and bugs were enclosed in screen-topped pill boxes

TABLE 14. The Effect of Contact Moisture on the Gain in Weight of Individuals of *Leptocoris trivittatus* at Two Temperatures.

	Condition	Number	Dry weight in milligrams	Initial water content in per cent	Final water content in per cent	Per cent gain in weight
Temperature 2°C.	Active	10	12.45±0.61	64.87±1.16	66.48±1.18	4.75—0.86
	Fair	10	13.05±0.63	58.44±0.72	59.42±0.69	4.76±0.84
	Poor	10	11.41±0.51	51.51±0.76	53.23±0.81	3.90±0.77
Temperature 6°C.	Active	10	11.43±0.98	65.64±0.93	70.06±0.72	14.62±3.22
	Fair	10	11.89±0.75	58.59±0.81	67.55±1.76	29.50±6.06
	Poor	10	13.63±0.76	52.84±1.12	56.14±1.60	7.82±1.88

over distilled water in desiccators. The dry leaves had been allowed to stand in the laboratory all winter and the wet leaves were prepared by soaking dry samples in tap water. The bugs were placed in the center of the tins to insure a good contact with the leaves as was possible. Table 15 shows that the insects which were in contact with the wet leaves gained

TABLE 15. *Leptocoris trivittatus* Exposed for Eight Days in Dry and Wet Leaves at 100 Per Cent Relative Humidity.

Condition	Per Cent Change in Weight
Dry leaves at 6°C.	−5.25±2.36
Dry leaves at 2°C.	−3.05±0.24
Wet leaves at 6°C.	+9.52±1.63
Wet leaves at 2°C.	+6.05±0.30

weight although the others in dry leaves lost weight. The gain in weight at the two temperatures differed as in the previous experiment except that the difference was not as great. The small number of experimental animals and the chance of poor contact with wet leaves make it impossible that some

of the insects did not gain the maximum amount possible in the time of the experiment.

It should be noticed that the insects in dry leaves lost weight at either temperature in spite of the fact that they were in a saturated atmosphere. On the contrary, the leaves in which they were exposed did take up water. The actual gain in the weight of the leaves cannot be reported because of an accident.

In the previous experiment it was observed that the bugs exposed in dry leaves lost weight. At first it was supposed that the dry leaves, because of their hygroscopic nature, had withdrawn water from the insects. In order to test this supposition, another series of hibernating box-elder bugs were exposed for five days to a saturated atmosphere at 6°C., 10°C. and 15°C. The technique used was the same as that employed in the desiccation experiments which were discussed in a previous section. The insects lost weight at all three temperatures: 2.0 ± 0.13, 5.09 ± 1.34, and 11.89 ± 1.98 per cent at 6°C., 10°C. and 15°C. respectively. The effect of temperature was to increase the per cent loss of weight as the temperature became higher. There was a lower percentage weight loss at 6°C. than there had been in the previous experiment at the same temperature with dry leaves. This indicates the possibility that the leaves might have increased the rate of evaporation from the insects. It was not possible, however, to be sure that the change in weight was all due to the loss of water. The insects were collected outside in the spring and were not very uniform with respect to the proportions of water in their bodies. Nevertheless, it was known that only a very small percentage of dry matter is used up after exposures of several weeks at temperatures of 2°C. and 6°C.

The effect of contact moisture on the gain in weight of dormant prepupae of the green blowfly has been investigated with regard to the physiological condition of the insects. Three lots of prepupae were placed in contact with moist cheese cloth for eight days at 6°C. One lot of insects had been desiccated for one month in dry sand at 2°C. The second group had been desiccated for one week under the same conditions. The third physiological type of prepupae was studied as soon as they had completed their feeding. The results of this study are presented in Table 16. The first group had the lowest initial water content and likewise showed the greatest gain in weight.

TABLE 16. The Effect of Contact Moisture at 6°C. on the Change in Weight of *Lucilia serricata* Prepuae.

	Number	Initial water content in per cent	Final water content in per cent	Per cent change in weight
Prepupae conditioned one month in dry sand at 2°C.	10	68.16±1.22	72.30±0.61	14.02±4.65
Prepupae conditioned one week in dry sand	8	72.88±1.17	74.18±0.69	5.11±1.56
Prepupae taken directly from food	8	77.16±0.49	75.45±0.05	−2.16±0.05

The second group gained a little weight, but the proportion of water at the start was apparently too high for them to take up much water. The third group actually lost water even though the insects were in contact with a moist medium in a saturated atmosphere. The final proportion of water in the three groups is very similar. This condition suggests the idea that at this combination of temperature and moisture a proportion of about 74 per cent water is maintained. The prepupae would have pupated under these conditions if they had remained longer, although a certain percentage of dry individuals will pass through a diapause at this temperature.

In order to determine the effect of a saturated atmosphere alone a study was made of a series of prepupae which had been desiccated in dry sand and 2°C. for one month. The insects were placed over distilled water at 6°C. for one week. The change in weight was practically negligible. There was a loss of 0.5 per cent of the total weight.

Hibernating larch sawfly larvae were secured from Mr. Orr, formerly forest entomologist at the Experiment Station of the University of Minnesota. These larvae were exposed to contact moisture at 2°C., and also to a saturated atmosphere at the same temperature. The naked larvae lost weight in a saturated atmosphere and gained a little weight when they were in contact with water. The combination of larvae and cocoons showed a gain in weight under both moisture conditions, but this gain did not affect the larvae. The addition of water was limited to the cocoons and did not affect the larvae within them. The effect of the cocoon is shown in Table 17. In all cases in which the larvae were treated in their cocoons,

TABLE 17. Comparison of the Percentage Water Content of Sawfly Larvae and Cocoons under Several Conditions.

Average initial water content	Average final water content	Treatment of sawfly larvae and cocoons
64.3	....	Sample of larvae taken at random from material used in experiment
13.5	....	Sample of cocoons taken at random from material used in experiment
64.2	61.7	Larvae exposed without cocoons to a relative humidity of 20 per cent
64.0	63.8	Larvae exposed with cocoons to a relative humidity of 20 per cent
13.9	8.9	Cocoons of larvae exposed to a relative humidity of 20 per cent
59.7	63.6	Larvae without cocoons exposed to contact moisture
63.7	63.2	Larvae with cocoons exposed to contact moisture
59.6	58.4	Larvae without cocoons exposed to 100 per cent relative humidity
63.6	63.3	Larvae with cocoons exposed to 100 per cent relative humidity
14.1	23.9	Cocoons exposed to 100 per cent relative humidity
13.7	42.4	Cocoons exposed to contact moisture

the change in the proportion of water was found in the cocoon and not in the larvae. For example, the cocoons exposed to contact moisture changed in water content from 13.7 to 42.4 per cent, and yet the larvae showed no change. From this experiment it is evident that some insect cocoons are of equal importance in the conservation of moisture and in the prevention of excessive hydration. The latter is very important in the life of the larch

sawfly because the larvae are often submerged in water for various lengths of time during the spring.

Moisture conditions which prevail in the spring may have an important place in the economy of insect life. At this time many species are either leaving their hibernation quarters, or they are transforming from one stage of development to another. In many cases this spring activation has been found to be dependent upon the presence of moisture. Breitenbecker (1918), Douglass (1928, 1933), Townsend (1926), Babcock (1927), Zwölfer (1930), and Cousin (1932) have all reported cases in which the presence of moisture, usually contact moisture, has been essential for the resumption of normal activity after dormancy. It has been proved by this investigation that the return to normal activity is hastened by a gain in water, and especially that the gain in water is dependent upon the actual contact of the insects with water in a liquid form. However, Breitenbecker (1918), Bodine (1921), and Buxton (1930) have demonstrated that some species can obtain moisture from saturated air. It is presumed that they all excluded the possibility of the condensation of water on the insects. Bodine demonstrated that the hibernating nymphs of *Chortophaga viridifasciata* would absorb increasing amounts of water as the temperature was raised. On the contrary, when adult *Leptocoris trivittatus* were studied in a similar manner, there was an increasing loss of weight as the temperature was raised. All of the observations reported in the literature as well as those made during this study agree that there is much less water taken up at low temperatures. The survival value of this phenomenon needs no additional discussion.

The theory of Townsend (1926) may be considered in the light of the present investigation. He concluded that the activity of an autolytic enzyme is essential for the breaking of dormancy in the codling moth. His data shows that the activity of the enzyme is dependent upon the water content of the tissues and the temperature. However, he did not measure the water content directly; it was estimated from the number of soakings which the larvae received. The optimum temperature was found to be 10°C. Soakings at about 0°C. and at the higher temperatures of 22°C. and 30°C. did not produce as rapid a recovery from dormancy as 10°C. Boyce (1931) has criticized the theory on the basis that the summer broods of codling moth larvae can pupate at temperatures above 22°C. This criticism is not particularly good because some individuals will enter a diapause during the summer and will not pupate until the following spring in spite of favorable temperatures. It seems more logical to believe that Townsend was dealing with the combined problem of water gain and water loss. The larvae soaked at the lowest temperature would not take up much water because of the low rate of metabolism of those dormant insects. It has been shown that other insects tend to take up water until a certain balance between water and dry material has been reached. It seems probable that about the same

amount of water would be gained by the larvae when they were soaked at each of the three higher temperatures. They were not exposed in saturated atmospheres, and consequently the amount of water evaporated from the larvae would increase in proportion to the increase in temperature. The net result would be that the larvae kept at 10°C. between soakings would actually maintain the highest proportion of water, the others at higher temperatures losing by evaporation much of the water gained by soaking. This explanation makes it seem possible that the enzyme activity is controlled directly by the water balance of the insects and not necessarily by an optimum low temperature of 10°C.

SUMMARY AND CONCLUSIONS

In this investigation of the rôle of water in insect hibernation several aspects of the problem have been studied. A survey of typical hibernation quarters was made with particular reference to the conditions of moisture which prevailed. A literature survey of the hibernacula of some economic insects was made to determine not only the nature of the hibernation quarters but also the stage of development of the hibernating insects. Experiments were performed with insects entering hibernation to discover whether or not there was any moisture preference exhibited. The resistance to desiccation of several species which hibernate with various degrees of exposure was determined. A series of experiments was carried out to show the relation of environmental moisture to the survival of insects exposed to low temperatures. Finally, dormant insects were studied to discover the manner in which water may be taken up at the advent of spring. The results of each study have been analyzed and the following conclusions have been drawn:

1. In Minnesota a high percentage of insect species are sheltered from a direct exposure to the low temperatures of winter. Hibernacula which offer protection, at least against sudden changes in temperature, may be found in logs, under fallen leaves, in the lower parts of plant stems, in the soil, and in other similar places. The protective value of all of these situations is increased by the presence of snow cover.

2. Insects are frequently found hibernating in places where the atmospheric moisture is at the saturation point. In fact, many species were found in actual contact with free water or encrusted with ice.

3. Some species of insects show a definite preference for a moist environment when they are stimulated by a falling temperature. It is believed that this reaction to moisture is partly responsible for the choice of hibernation quarters. It is unreasonable to suppose that insects can predict that a certain situation will offer protection from cold; however, the factors which protect a hibernaculum from the excessive evaporation of water may also influence the penetration of cold.

4. When insects are desiccated at a moderately low temperature the individuals of a species show a marked uniformity in the proportion of water in their bodies at death, although this minimum value may vary considerably from one species to another. The survival time during desiccation is more dependent upon the rate of loss of water and the initial water content than upon the lethal limit of desiccation. There was no consistent relation between the ability to conserve water and the amount of exposure to desiccation during the winter.

5. Atmospheric moisture influences the cold hardiness of insects by controlling the amount of water which is evaporated from the tissues. Either insufficient or excessive loss of water is harmful. The relation of either saturation deficiency or absolute humidity to cold resistance depends upon the time and the degree of desiccation.

6. Contact moisture influences the freezing of insects by the prevention of the protective phenomenon of undercooling of the tissues and possibly by a hydration of the tissues.

7. The breaking of dormancy is aided by the absorption of water by the insect tissues. The amount of water taken up is dependent upon the temperature and the physiological condition of an insect. At temperatures which induce quiescence, enough water may be gained to raise the proportion to the normal percentage. Insects in contact with water are better able to begin their spring activity when the temperature is favorable.

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